Spatiotemporal variability of adult Antarctic krill (*Euphausia superba*) lipids in relation to sea surface temperature and Chlorophyll *a*

by

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Declaration of originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge no material previously published or written by another person except where acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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All authors contributed to project design. NH and JAE performed laboratory analysis. NH completed all data analysis, wrote the manuscript and attended to reviews. PDN, SK, SN, N Hoem and PV assisted in manuscript editing.

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Dedication

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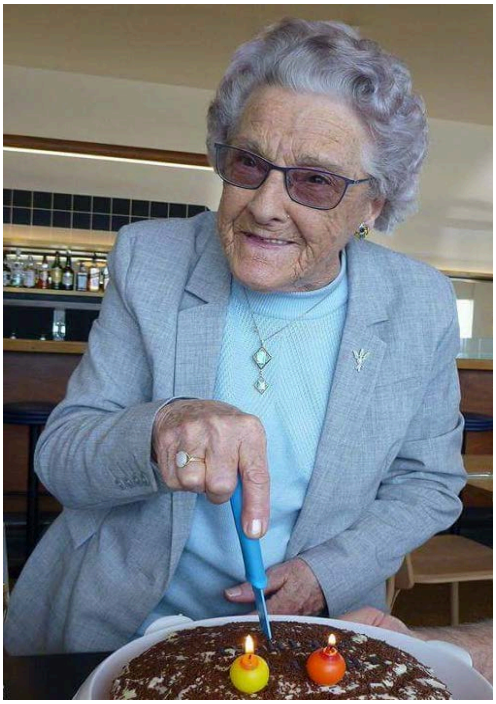

Glossary

2F – sub-adult female

2M – sub-adult male

3F – mature female

3F-G – gravid female

3F-S – spent female

3M – mature male

3x3 – 3 km x 3km pixel

8D – 8 day average

Area 48 – South Atlantic Ocean sector

Area 58 – South Indian Ocean sector

Area 88 – South Pacific Ocean sector

CCAMLR – Convention for the Conservation of Antarctic Marine Living Resources

Chl *a* – chlorophyll *a* (mg m^{-2})

DAG - diacylglycerol

DHA – docosahexaenoic acid (22:6n-3)

DM – dry mass

EPA – eicosapentaenoic acid (20:5n-3)

FAME – fatty acid methyl ester

FFA – free fatty acids

Fishery-derived samples – samples collected by/from the fishery

GC-FID – gas chromatography flame ionisation detector

GC-MS – gas chromatography – mass spectrometer

GPS – global positioning system

HC – hydrocarbons

LC-PUFA – long chain ($\geq C_{20}$) polyunsaturated fatty acids

MODIS - moderate resolution imaging spectroradiometer

MUFA – monounsaturated fatty acids

MSI – marine snow indicators

n-3 – omega 3

PCA – principal component analysis

PL – phospholipids

PUFA – polyunsaturated fatty acids

RRS – remote sensed reflectance wavelengths

SD – standard deviation

SDA – steariadonic acid (18:4n-3)

SE - steryl esters

SFA – saturated fatty acids

SG – South Georgia (Sub-Area 48.3)

SO – Southern Ocean

SOI – South Orkney Islands (Sub-Area 48.2)

SST – sea surface temperature

ST – sterols

TAG – triacylglycerols

TLC-FID – thin layer chromatography – flame ionisation detector

TL – total lipid (mg)

TL DW – total lipid dry mass (mg g^{-1})

TSE – total solvent extract

TSN - total non-saponifiable neutral lipids

WAP – West Antarctic Peninsula (Sub-Area 48.1)

WE – wax esters

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Abstract

Lipids are key biochemicals that form both cell membranes and energy stores. Lipids are of particular importance in energy poor environments where animals require stores to survive long periods of food shortage. In the Antarctic, food availability is dominated by extreme seasonal shifts in the environment and energy rich food is scarce for a substantial period of time each year. Antarctic krill (*Euphausia superba*) have adapted to have large lipid stores (over a third of their dry weight) during winter for this reason. Krill are a key species in the Antarctic environment; their biomass links lower and higher trophic levels and forms the main energy conduit for the system. Krill feed on diatoms, dinoflagellates and other algal species year-round, resulting in high omega-3 polyunsaturated fatty acids which are essential for krill health, growth and reproduction. Krill-derived omega-3 containing products (particularly eicosapentacnoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3)) are sold as nutraceuticals for human consumption. Krill oil tablets (sold as an omega-3 supplement) are now the fastest growing nutraceutical globally.

Understanding the krill life cycle is hampered by the restricted nature of scientific sampling. Knowledge of krill diet and krill lipid dynamics is lacking for the Indian and Pacific Ocean sectors, as most studies have focused on the South Atlantic Ocean sector where the krill fishery is based. Most scientific research voyages are conducted during summer months and all scientific studies are restricted in their spatial and temporal scale. Information on krill recruitment and reproduction in the Indian and Pacific Ocean sectors is also not as developed as in the South Atlantic, where fishery-derived samples are also available.

A major gap in current ecosystem models is the link between environmental drivers (such as upwelling of nutrients, sea surface temperature and height, sea ice extent and thickness and salinity) and their impact on primary production and therefore food availability during extreme

seasonal shifts in Antarctica. One way of measuring these environmental drivers is through remote-sensing via satellite, which can gather data over large geographic areas and over long timeframes. Satellite-derived data for biological and ecological measures is still developing as a tool for oceanographers and other end users. However, one area of growing importance is in the use of ocean colour data which can be converted into chlorophyll *a* concentrations (a proxy for primary production) via a standard algorithm. By linking the GPS locations of commercial krill harvesting, and therefore krill swarms, to environmental data obtained through remote-sensing from the same date, the relationship between the environment krill live in and their biochemical composition can be examined in ways not previously explored.

My study used samples collected by a member of the krill fishery, Aker BioMarine, over a continuous three-year period in the South Atlantic Ocean to look at the seasonal and interannual trends in krill total lipids and lipid classes (such as those used for energy storage and the structure and function of cells). This dataset is unprecedented in its seasonal and spatial coverage in the South Atlantic Ocean. This study has been able to establish the sinusoidal shape of the seasonal and interannual trend in krill total lipids and its associated lipid classes. No samples from the fishery were available from other sectors. These South Atlantic Ocean krill samples were contrasted to krill samples collected from scientific expeditions in the other two ocean basins surrounding Antarctica (Pacific and Indian Oceans). Krill diet was investigated at a regional scale during the crucial late-summer spawning period. Results from my study revealed that krill diet varies between ocean basins, with Indian Ocean krill showing a distinctly different diet to Pacific and Atlantic Ocean krill, during the late-summer.

The fishery-derived samples were also related back to environmental data collected via satellite, for both chlorophyll *a* and sea surface temperature, to investigate if environmental drivers influenced krill lipid biochemistry. This study showed that both sea surface temperature and chlorophyll *a* concentrations (derived from ocean colour data) can be related to krill lipid

and fatty acid dynamics. Krill lipid composition and content were shown to be correlated to these environmental factors through simple models. The combination of results from this study will help fill the data gaps in ecosystem models and enable better determination of krill diet, recruitment and reproduction in all ocean basins surrounding Antarctica. These advances in krill knowledge will help improve fishery management policies.

Chapter 1: Introduction

Background Krill Information

Antarctic krill (*Euphausia superba,* hereon krill*)* are a keystone species in the Southern Ocean (SO) ecosystem. Mature adult krill range from 30 to over 60 mm in length (Standard length 1; Kirkwood, (1982)), with a life span of 5-6 years (Siegel, 1987) in the wild. Krill are found at latitudes as far north as 50 °S (near South Georgia) to the Antarctic continental edge and can survive in sea temperatures from -1.5 ℃ to +5 ℃ (Miller and Hampton, 1989, Atkinson et al., 2008). Krill are generally found in greater densities and are more abundant in high nutrient waters that are rich in phytoplankton (Siegel, 2005). Krill can be found from the surface of the ocean to depths of 4,500 m, but are more often seen in the upper 200 m of the water column (Morris et al., 1984, Schmidt et al., 2014).

Krill reproduction and recruitment can be highly variable from year to year (Yoshida, 2009, Kawaguchi, 2016) and depends largely on feeding conditions. The availability of sea ice algae, which is directly related to sea ice extent, is known to influence juvenile recruitment in krill (Atkinson et al., 2002, Virtue et al., 2016, Schaafsma et al., 2017). During the productive spring and summer seasons, krill populations grow from the new recruitment of juveniles before diminishing over the harsher winter season (Kawaguchi and Satake, 1994, Siegel and Loeb, 1995, Wiedenmann et al., 2009). Krill also aggregate to form swarms that can be up to 100 km^2 (Tarling et al., 2009a). These swarms can range in densities from 100 krill m^{-3} to over 1,000 krill m^{-3} (Klevjer et al., 2010). The sex ratio of individuals in swarms can vary from 100% male to 100% female in swarms during the reproductive spawning season in late summer to 50:50 male:female during winter (Atkinson et al., 2006, Tarling et al., 2016a). The patchy distribution and vast geographic range of krill makes it difficult to provide robust estimates of krill biomass and production (Nicol and Endo, 1999). Circumpolar estimates place krill

biomass at roughly 80-200 million metric tons (Demer, 2004, Atkinson et al., 2009). This large biomass within the SO ecosystem makes krill both a keystone species and the center of the wasp-waisted ecosystem, where energy is funneled from lower to higher trophic levels through a single species (Figure 1.1).

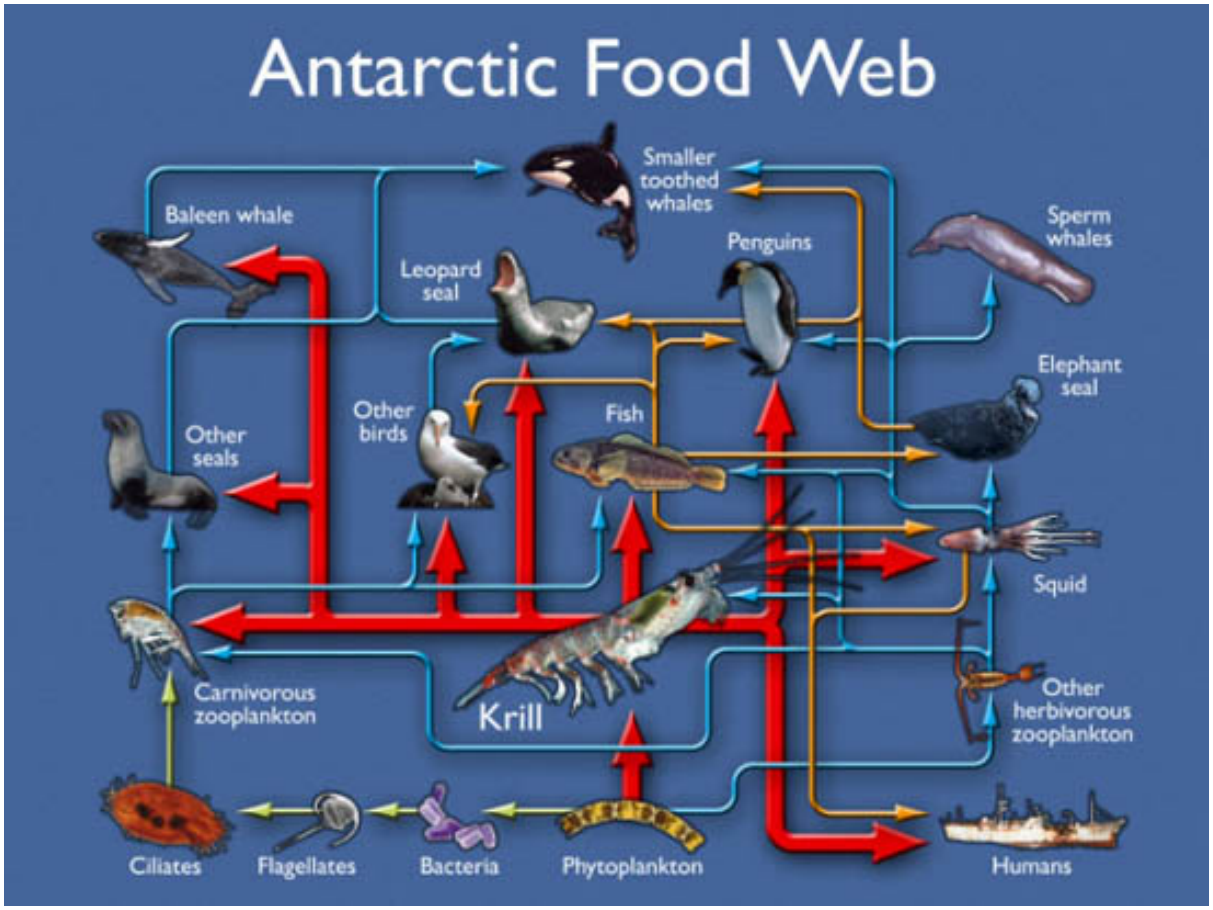

Figure 1.1: The Antarctic Food Web http://www.classroomatsea.net/JR161/about.html accessed 2/8/16.

Most of the charismatic megafauna associated with Antarctica such as seals, penguins and whales, as well as fish, squid and seabirds rely on krill as a primary food source (Hill et al., 2006, Murphy et al., 2007, Ward et al., 2012a). Krill are often an indirect secondary food source as well, due to it being an abundant energy-dense nutrient source (Hagen et al., 2001) in the ecosystem. Krill form the key link between primary production and higher trophic levels in Antarctica (Everson, 2008). Most apex predators in the SO tend to breed during the austral

summer, due to the higher abundance of available prey at this time (Bryden, 1983, Trivelpiece et al., 2011, Watts and Tarling, 2012). Similarly, krill also spawn at this time as they are feeding on the increased levels of primary production due to the spring/summer algal blooms (Garabotti et al., 2005; Vernet et al., 2008). Krill become more energy-dense throughout summer due to their grazing on the increased levels of primary production, which in turn increases their fat levels. During the other times of the year, marine predators forage at dynamic oceanographic features where increased levels of primary production (and hence krill) can be found year-round (Charrassin et al., 2002, Heerah et al., 2013).

Krill diet varies greatly. Krill consume phytoplankton, predominantly diatoms and dinoflagellates, which are high in chlorophyll *a* (a green pigment) and cause krill digestive glands to be green throughout periods of high feeding (Virtue et al., 1993a; Yoshida et al., 2009). They also consume zooplankton, such as copepods, as well as bacteria, marine snow and sea ice microbiota (Passmore et al., 2006, Kohlbach et al., 2015). Krill diet is also known to shift with environmental conditions. Krill are known to be cannibalistic during harsher winter conditions (Atkinson et al., 2002, Ju and Harvey, 2004, O'Brien et al., 2011, Virtue et al., 2016, Meyer et al., 2017) and can be almost entirely herbivorous during good summers (Virtue et al., 2011; Ericson et al., 2018a). Krill diet has been studied using various techniques including DNA sequencing (Passmore et al., 2006), microscopy (Töbe et al., 2010) and signature fatty acid analysis (Virtue et al., 1993a, Virtue et al., 2000, Phleger et al., 2002, Schaafsma et al., 2017). Such studies are often restricted by the number of samples and the season and location of sample collection. Thus, how krill diet changes in time and space and especially how krill diet will be impacted by climate change is still an emerging area of research (Kawaguchi et al., 2011, Ericson et al., 2018b). It has been suggested there will be a shift from energy rich (e.g. krill) to energy poor species (e.g. salps) in polar regions under climate change (Loeb et al., 1997, Atkinson et al., 2004). As primary production and microbial community

assemblages shift with climate change (Deppler and Davidson, 2018; Hancock et al., 2018), energy transfer at lower trophic levels will be affected. This shift in krill diet will in turn have major implications for the transfer of energy up to higher trophic levels (Kattner et al., 2007).

There has been a large focus on krill-centric studies in the Antarctic because of krill's large biomass and central role in the wasp-waisted SO ecosystem. From the Discovery voyages in the early 1900s (Hardy, 1928, Deacon et al., 1939) to the more recent acoustic surveys in the 2000s (Nicol et al., 2000a, Demer, 2004, Jarvis et al., 2010), krill have been the focus of many large multinational expeditions (Nicol et al., 2010). However, most scientific expeditions are only able to sample and undertake voyages during the summer season due to logistical, financial, and time constraints. Because of this constraint on scientific voyages, there is only limited information available about their winter adaptations (Huntley et al., 1994, Kolakowska et al., 1994, Daly, 2004, O'Brien et al., 2011). The winter period will impact on all other aspects of krill life such as their metabolism, growth, reproduction and diet. Modern facilities, such as laboratories and aquariums (e.g. the Australian Antarctic Division's Krill Aquarium; Kawaguchi et al. (2010a)), have helped fill this gap by enabling the study of krill life history and their seasonal diet. Laboratory studies have also improved the understanding of krill genomics, krill aging and how micro-plastics impact krill. Aquarium use has also enabled ocean acidification studies to be undertaken; such studies cannot be performed *in situ* (Virtue et al., 1997, Yoshida et al., 2009, Kawaguchi et al., 2011, Kilada et al., 2017, Dawson et al., 2018, Ericson et al., 2018b). These facilities, however, cannot replicate the large regional and interannual environmental stressors impacting krill.

Lipids and Krill Biochemistry

Lipids are biochemical building blocks that are key to the structure and function of living cells and can have multiple roles in organisms. Examples of lipids include fats and oils used in

energy storage (Hagen et al., 1996, Varpe et al., 2009, Yin et al., 2016), as well as waxes, certain vitamins (Burton and Ingold, 1986), hormones (Fernlund and Josefsson, 1972) and most of the non-protein membrane of all cells (Caraveo-Patiño et al., 2009, Tancell et al., 2012). Lipids are classified into lipid classes, such as: polar lipids (includes phospholipids, PL) and neutral lipids (triacylglycerols (TAG), wax esters (WE), sterols (ST), free fatty acids (FFA), hydrocarbons (HC) and diacylglycerols (DAG)). The major lipid classes are predominantly made up of smaller building blocks known as fatty acids which themselves come in various forms. Fatty acids can be: without any carbon-carbon double bonds, so called saturated fatty acids (SFA), or can be unsaturated fatty acids, which can have either one carbon-carbon double bond (monounsaturated fatty acids (MUFA)) or multiple carbon-carbon double bonds, polyunsaturated fatty acids (PUFA). Long-chain ($\geq C_{20}$) PUFA (LC-PUFA) are essential for health and survival in organisms, particularly the omega-3 (n-3) LC-PUFA which are required for lipid derived cell signaling, cell membrane fluidity, reproduction and growth (Kolakowska et al., 1994, Ross and Quetin, 2000, Yoshida et al., 2011, Kawaguchi, 2016). The variability of lipid and fatty acid content and composition in krill has been related to sex, developmental stage, nutrient conditions and nutritional requirements (Clarke, 1984, Saether et al., 1985, Pond et al., 1995, Skerratt et al., 1995, Mayzaud, 1997, Virtue et al., 1997, Mayzaud et al., 1998, Atkinson et al., 2002, Ju and Harvey, 2004, Alonzo et al., 2005, Yoshida et al., 2011).

Most marine organisms use TAG and/or WE as their primary form of energy storage and may store their n-3 LC-PUFA within these stores (Wakefield et al., 2008, Mayzaud et al., 2011, Pond et al., 2014). Krill can also use PL as an energy store (Itonori et al., 1991, Hagen et al., 1996, Yin et al., 2016), in addition to its structural role, and their n-3 LC-PUFA are primarily found in this lipid class (Kolakowska et al., 1994, Mayzaud et al., 1998, Hill, 2013).

The fatty acids found within an organism are accumulated through dietary inputs (Iverson et al., 2004), as well as being broken down and metabolized into other fatty acids

(Huntley et al., 1994). Some fatty acids cannot be broken down or converted in higher level organisms and can therefore be traced and quantified as a biomarker of their dietary input (Volkman and Nichols, 1991, Iverson et al., 2004) through thin-layer chromatography and signature fatty acid analysis. Signature fatty acid analysis allows us to interpret the links between trophic levels and to also quantify the prey items seen in the diets of predator tissues. These fatty acid biomarkers can show seasonal variability due to environmental and energy demands of various organisms. For example, krill have seasonal fluxes in their total lipids (up to 40% dry mass at the start of winter (Falk-Petersen et al., 2000, Atkinson et al., 2002)) and in their n-3 LC-PUFA, which increase with the phytoplankton blooms in the austral summer (Ericson et al., 2018a). Tracing the energy flow in the Antarctic food web is particularly important due to the relative simplicity and small number of trophic levels within the system (Figure 1.1) compared to most other marine ecosystems. Any breakdown of energy flow within the food web has far reaching effects, as the alternative pathways are often few and do not contain the required nutrients to support higher trophic levels if the original pathway is not restored. For example, higher trophic level predators require krill-derived n-3 LC-PUFA for their health, growth and reproduction. The alternative trophic pathway, mainly through salps, is lipid and therefore n-3 LC-PUFA poor (Loeb et al., 1997, Atkinson et al., 2004).

Most of the lipids in the SO originate from primary producers and in particular the essential n-3 LC-PUFA; eicosapentaenoic acid (20:5n-3, EPA), docosahexaenoic acid (22:6n-3, DHA) and stearidonic acid (18:4n-3, SDA), which are diatom and dinoflagellate markers respectively (Nichols et al., 1986, Nichols et al., 1988, Schmidt et al., 2012, Kohlbach et al., 2017). If primary production alters on a large scale in the SO due to an environmental shift, then all of the consumers up the trophic links will be influenced by that change, particularly in their fatty acid biomarkers. Hence, a decrease in primary production can be linked to a decrease in n-3 LC-PUFA levels in primary consumers such as zooplankton (Phleger et al., 1998, Hagen

and Kattner, 2014, Turner, 2015, Kohlbach et al., 2018). These n-3 LC-PUFA, particularly EPA and DHA, are also the main oil parameters targeted by the commercial krill fishery (Butler, 2007, Hill, 2013, Schutt, 2016).

Krill Oil and the Krill Fishery

The Antarctic krill fishery began in the late 1960s, and is governed by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR), a world leader in sustainable fishing practices (Nicol and Endo, 1999, CCAMLR, 2017). During the 2000s the fishery expanded to include the production of commercially processed krill oil (Nicol et al., 2012). Krill oil recently emerged internationally as an important alternative to fish oil as a source of n-3 LC-PUFA, as a nutraceutical product for human consumption. Krill n-3 LC-PUFA are mainly present in the phospholipid form, whereas other commercially available n-3 LC-PUFA supplements, such as fish and squid oil, are generally in the conventional TAG form. Krill oil also contains natural antioxidants such as astaxanthin, which krill themselves contain. With an estimated value of $AUD 3.3 million/tonne (Kawaguchi, pers. comm.), several new krill oil products are now in the top five omega-3 supplements sold within Australia (Schutt, 2016). The trend of commercial krill fishing operators seeking to expand into this niche is growing rapidly (CCAMLR, 2012, Nicol et al., 2012). The impacts of this expanding fishery on the SO ecosystem and krill biomass is not well understood, particularly in sectors outside of the South Atlantic Ocean (Area 48, Figure 1.2). In 2016, the Chinese started an exploratory krill fishery in East Antarctica, within the Indian Ocean Sector (Area 58, Figure 1.2), but knowledge of krill diet, health, growth, recruitment and reproduction in this region is mostly lacking (Nicol et al., 1992, Mayzaud et al., 1998, Pakhomov, 2000, Swadling et al., 2010, Virtue et al., 2010). The Pacific Ocean Sector (Area 88, Figure 1.2) has rarely been fished and there is even less available data on krill from this region.

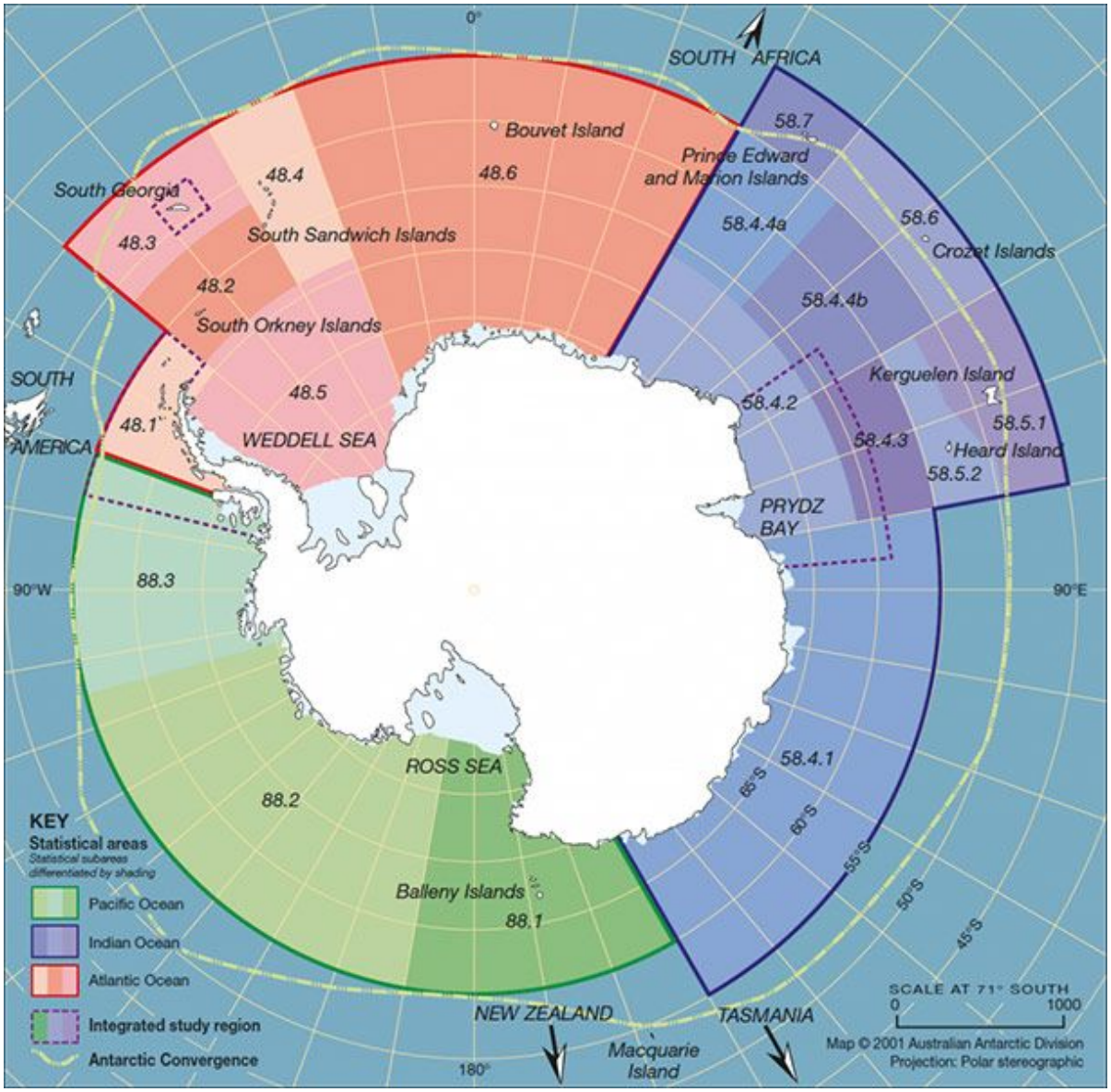

Figure 1.2: CCAMLR Statistical Areas and Sub-Areas from http://www.antarctica.gov.au/magazine/2001-2005/issue-1-autumn-001/international/ccamlr - the-first-20-years accessed 17/2/16.

CCAMLR currently sets krill fishing catch limits based on precautionary ecosystem models to ensure the health of the entire Antarctic ecosystem. As lipid storage is vital for krill reproduction and recruitment (Yoshida et al., 2011, Kawaguchi, 2016), providing CCAMLR with data that includes krill lipid dynamics throughout all seasons and from different regions will be useful to help fill gaps in these models.

Due to the ability of the fishery to cover large geographic areas and to harvest krill year-round, the utilisation of samples from the krill fishery for scientific research purposes is becoming more common (Kawaguchi and Nicol, 2007, Hill, 2013, Descamps et al., 2016, Nicol and Foster, 2016, Tarling et al., 2016a). Gaining knowledge and samples of krill from the fishery is a cheaper alternative to scientific voyages. Aker BioMarine, a Norwegian krill fishing company, lands over half the total global krill catch for use in aquaculture and human consumption (CCAMLR, 2017). The company harvests 24 hours a day, from December to September every year, using two high technology vessels that are fitted with a mid-water continuous pumping system (Siegel, 2016). Aker BioMarine harvests krill from the South Atlantic Sector (Area 48), specifically the West Antarctic Peninsula (WAP, Sub-Area 48.1), the South Orkney Islands (SOI, Sub-Area 48.2) and South Georgia (SG, Sub-Area 48.3). Fishing companies generally have a greater geographic coverage of krill in the region than even some large multinational expeditions such as the CCAMLR 2000 survey (Nicol et al., 2000a, Demer, 2004). Fisheries-based research can cover a wide area and krill samples can be collected for much of the year, unlike scientific voyages.

Remote-Sensing Environmental Conditions

Another alternative to traditional scientific voyages to gain insights into the Antarctic environment is the use of data from remotely operated sensors, such as satellite-based instrumentation. Satellites are able to measure multiple environmental variables simultaneously including, but not limited to: sea surface temperature (SST), sea surface height, fluorescence, ocean colour, sea ice extent and thickness, wind speed and direction, and chlorophyll *a* concentration (a proxy for primary production) (Moore and Abbott, 2002, Johnson et al., 2013, Zeng et al., 2016, Kahru et al., 2017). Unfortunately, the use of such remote-sensing techniques to gain environmental data, such as ocean colour, is incredibly difficult in polar regions due to cloud cover, sea ice reflectance (albedo effect) and the sun

angle in winter (IOCCG, 2015). Satellites can, however, collect data over a range of geographic areas (small 1 km x 1 km pixels to large 100 km x 100 km boxes (Moore and Abbott, 2002, Zeng et al., 2016)) and time periods (as short as 5 minutes and as long as 10+ years, (IOCCG, 2015, Kahru et al., 2017)). Such broad data collection allows for multiple scales of environmental data to be used in relation to other variables, such as biological factors.

Relating biological data to environmental data from satellites is still in its infancy, particularly in polar regions. The use of accessible remote-sensed data is still not common for biologists, ecologists and physiologists (Moore and Abbott, 2002, Rayner, 2003, Mackey et al., 2012, Tarling et al., 2018). One of the few often-used remotely-sensed pieces of biological data is ocean colour data, which can be converted into chlorophyll *a* concentrations using algorithms such as those reported by Johnson et al. (2013). Greater expertise in biological remote-sensing data could rapidly increase knowledge and understanding of how climate change impacts the biology and ecology of polar regions. This includes the effect of the reduction of sea ice extent on juvenile krill diet and recruitment through the loss of sea ice algae (Loeb et al., 1997, Atkinson et al., 2004).

In terms of climate change, how krill will deal physiologically and ecologically with warming oceans and ocean acidification is a developing area of research (Kawaguchi et al., 2011, Kawaguchi et al., 2013, Loeb and Santora, 2015, Tarling et al., 2016b, Ericson et al., 2018b, Atkinson et al., 2019, Ericson et al., 2019a). Studies on the effects of climate change and ocean acidification on krill have reported negative effects on embryonic development (Kawaguchi et al., 2011), but negligible effects on krill lipid dynamics both in terms of ocean warming (Brown, 2010) and ocean acidification (Ericson et al., 2018b and 2019a) when fed a standard algal diet. Krill diet, however, may be impacted into the future; particularly if warming or acidification changes the primary production community composition such as in Deppeler and Davidson (2017), or by a microbial community assemblage change as in Hancock et al.

(2018). How such changes will impact on krill diet is currently unknown. Further controlled experimental studies need to be conducted to investigate how increased temperatures, different primary production and microbial community compositions and ocean acidification will interact and affect the content and composition of krill lipids, with emphasis on the key n-3 LC-PUFA in all life stages of krill.

Aims and Structure of Thesis

The aims of my research are to provide a synthesis on the biochemistry and inferred diet of krill over large spatial and temporal scales. Krill lipid dynamics and krill diet was related to remotely-sensed environmental data. Specifically, the aims were to:

I. Investigate seasonal and interannual variations in krill by using total lipid and lipid class content and composition. An investigation into the content and composition of krill lipid and lipid classes using samples obtained from the krill fishery in the South Atlantic Ocean is presented in Chapter 2. This study is the first detailed examination of both the total lipid and lipid classes of krill across all seasons, spanning three consecutive years.

II. Examine the total lipid, fatty acid and sterol content and composition of whole krill, as well as from their digestive glands and stomachs, within all three of the ocean basins surrounding Antarctica (Pacific, Indian and Atlantic Ocean sectors). Chapter 3 presents results on krill diet, particularly as determined via analysis of the neutral lipid fraction, and it's variations at a regional scale during the crucial late-summer spawning period.

III. Explore whether the use of remotely-sensed environmental data can be linked to krill lipid dynamics. Specifically, sea surface temperature and chlorophyll *a* concentrations will be used, as they are known to be related to krill diet and can be measured via satellite instrumentation. This study combines and applies these varied methodologies within the polar region to show how end users might utilise this information. Results of this research are presented in Chapter 4.

In Chapter 5 the results from the combined chapters are synthesized into a General Discussion on the spatiotemporal variability of krill lipid dynamics and how krill diet (and therefore their dietary-derived lipids) might be affected by present day environmental parameters.

Outcomes presented in the individual chapters and overall thesis will be important for and directly relevant to krill fishing companies, CCAMLR, non-government organisations and Antarctic ecologists. The results are also relevant for ongoing management of the krill fishery by providing real-world examples of potential feedback management data. This includes provision of a baseline for these key biochemical and environmental parameters, and the possible effects of climate change to krill lipid dynamics.

Additional outcomes such as co-authored papers, conference presentations and scientific outreach from this project are recorded in Appendix 2.

Chapter 2: Seasonal and interannual variation in the lipid content and composition of *Euphausia superba* Dana, 1850 (Euphausiacea) samples derived from the Scotia Sea fishery

This chapter has been published:

Hellessey N, Ericson JA, Nichols PD, Kawaguchi S, Nicol S, Hoem N, Virtue P (2018). Seasonal and interannual variations in the lipid content and composition of *Euphausia superba* Dana, 1850 (Euphausiacea) samples derived from the Scotia Sea krill fishery. *Journal of Crustacean Biology*, doi:10.1093/jcbiol/ruy053.

ABSTRACT

The Antarctic krill (*Euphausia superba* Dana, 1850) is an important trophic link between phytoplankton and higher trophic levels. Knowledge of the lipid biochemistry of krill assists in understanding their seasonal biology and predicting their responses to ecological changes. We collected daily samples of krill from a commercial fishing vessel operating in the Atlantic Sector of the Southern Ocean from 2014 to 2016. We analysed the total lipid content of the krill and the relative distribution of lipid class levels to examine seasonal trends. Krill total lipid content varied significantly within and between seasons and sexes. An annual sinusoidal trend was seen in total lipid content with the highest values in autumn and the lowest in spring (average 380 and 87 mg g^{-1} dry mass, respectively). Total lipids in krill increased during summer, peaking in autumn, with the total lipids in winter individuals decreasing towards spring. The relative distribution of lipid class levels varied between season and year. Levels of triacylglycerol showed the same seasonal trend as total lipid content, whilst phospholipid showed the inverse trend indicating the contrasting roles of these two dominant lipid classes. These data provide high-resolution information on the seasonality of krill lipid content and composition. This information has both ecological and commercial utility.

INTRODUCTION

Antarctic krill (*Euphausia superba* Dana (1850)) is a keystone species in the Antarctic ecosystem as many of the marine predators in the region consume it due to its high abundance and nutritional value. Krill have high lipid (oil) content (Falk-Petersen et al., 2000, Iverson et al., 2004) when compared to other prey species such as myctophid fishes (Phleger et al., 1997, Phleger et al., 1999). Lipids are an important energy reserve for krill (Falk-Petersen et al., 1981), as well as being necessary for cell membrane structure and function. The omega-3 long-chain ($\geq C_{20}$) polyunsaturated fatty acids (n-3 LC-PUFA), largely eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), are required for reproduction and growth in krill (Clarke, 1980, Quetin et al., 1994, Mayzaud, 1997, Ross and Quetin, 2000, Tarling et al., 2009b) and animals at higher trophic levels (Suhr et al., 2003, Wakefield et al., 2008, Caraveo-Patiño et al., 2009, Mayzaud et al., 2011, Trivelpiece et al., 2011). Lipid content in krill has been related to sex, developmental stage, nutrient conditions, and nutritional requirements (Clarke, 1984, Virtue et al., 1997, Mayzaud et al., 1998, Ju and Harvey, 2004). Krill are primarily herbivores feeding on phytoplankton in the summer and sea-ice algae and associated organisms in the winter (O'Brien et al., 2011, Virtue et al., 2016, Schaafsma et al., 2017). They can also be carnivorous and are known to consume copepods (Kohlbach et al., 2017, Schaafsma et al., 2017) and may even become cannibalistic in harsher winter seasons (Ju and Harvey, 2004, Schaafsma et al., 2017). These changes in krill diet can be detected in their lipid content and composition (Clarke, 1984, Kohlbach et al., 2015, Schaafsma et al., 2017).

Understanding the seasonal cycle of krill lipids and lipid storage mechanisms will assist in better understanding krill life history (Quetin et al., 1994, Falk-Petersen et al., 2000, Yoshida et al., 2011). The role of lipids in krill during periods of limited food supply is unclear due to a lack of high-frequency year-round sampling, and in particular, adequate winter sampling (Ikeda and Dixon, 1982, Marschall, 1988, Hagen et al., 1996, Atkinson et al., 2002, Kohlbach

et al., 2017, Schaafsma et al., 2017, Kohlbach et al., 2018). Many questions on the key biology and ecology of krill remain unanswered because research in the Antarctic is logistically difficult and costly, particularly in winter. There is conflicting evidence as to whether krill store lipid for overwintering (Ikeda and Dixon, 1982, Marschall, 1988, Virtue et al., 1993b, Quetin et al., 1994, Hagen et al., 1996, Atkinson et al., 2002, Schaafsma et al., 2017) or whether they use their body lipid and protein and shrink in response to food shortage (Ikeda and Dixon, 1982, Nicol et al., 1992, Sun et al., 1995, Alonzo and Mangel, 2001, Tarling et al., 2016a). Better data from krill collected during winter will be critical in clarifying their over-wintering strategies and fishery samples have already contributed to this effort (Tarling et al., 2016a, Kim, 2014).

Lipid storage is the key to reproductive success in krill (Clarke and Morris, 1983, Cuzin-Roudy and Buchholz, 1999, Ross and Quetin, 2000). The cost of reproduction is also one of the main parameters involved in the formulation of an energy budget (Clarke and Morris, 1983, Perissinotto et al., 2000, Ross and Quetin, 2000). Female krill store lipids in their eggs prior to spawning and larval krill require these lipid stores to survive their initial growth and development (Clarke, 1980, Atkinson et al., 2002, Tarling et al., 2009a, Yoshida et al., 2011). Reproductive success and recruitment in krill populations are known to fluctuate markedly from year to year (Ross and Quetin, 2000). Understanding the causes of these variations in reproductive output requires samples collected at regular intervals throughout the year. Whilst regular sampling is possible near research stations in Antarctica, long-term open-ocean scientific sampling has proved difficult. Krill fishing vessels, however, operate year-round and are a potential source of krill in all seasons (Nicol et al., 2012). Samples from the krill fishery can provide a valuable insight into annual, seasonal, and short-term changes in krill biological parameters (Kim, 2014, Tarling et al., 2016a). Analyses of these samples can help inform the fishery on changes in krill habitat, ecology, physiology, and lipid biochemistry.

Krill has been commercially harvested for its oil, meal, and other components for the last four decades (Nicol and Foster, 2016). Krill fishing is the second-largest, single-species crustacean fishery in the world and the largest fishery in the Southern Ocean; 260,151 tonnes in 2016 (Nicol and Foster, 2016). The krill fishery has been located in recent years in the Scotia Sea, South Atlantic, namely FAO statistical Area 48 (Fig. 1A) (CCAMLR, 2017), although a small catch was taken from the Indian Ocean Sector in 2017 (CCAMLR, 2017). The fishing fleet migrates from the South Orkney Islands (SOI) (Sub-Area 48.2) in autumn, up to the West Antarctic Peninsula (WAP) (Sub-Area 48.1) as winter progresses then up to ice-free South Georgia (SG) (Sub-Area 48.3) when the WAP and SOI are either ice-covered or these fishing grounds have reached their target catch limits for the season (Fig. 1A, B).

The krill fishery produces meal and oil (Nicol and Foster, 2016). Antarctic krill oil has emerged over the past decade as an important and alternative source of omega-3 LC-PUFA for use in nutraceutical and pharmaceutical products for human consumption as well as in aquaculture and livestock feed (Schutt, 2016). In humans, omega-3 from krill oil may be more easily absorbed than fish oil because a substantial portion of the omega-3s are attached to phospholipids compared to the mainly triacylglycerol form found in fish oil (Kwantes and Grundmann, 2015). These two lipid classes dominate krill oil composition, but their seasonal and interannual changes are not clearly understood (Clarke, 1980, Pond et al., 1995, Mayzaud et al., 1998, Falk-Petersen et al., 2000, Atkinson et al., 2002).

We analysed fisheries-derived samples to gain insights into specific aspects of krill lipid production and storage, aiming to investigate the seasonal and interannual variation in the lipid content and lipid class content and composition of krill. We compared the lipid content and lipid class composition of krill caught at various fishing locations within CCAMLR (Convention on the Conservation of Antarctic Marine Living Resources) Area 48 throughout three years of continuous sampling.

METHODS

Sample collection and storage

One hundred individual krill of random sex, age, length, and mass were collected daily from a continuous live flow system on-board the Aker BioMarine fishing vessel, the FV *Saga Sea*, from 1 January 2014 to 11 September 2016 operating in CCAMLR Sub-Areas 48.1, 48.2 and 48.3 (Fig. 2.1A, B).

Krill samples were snap-frozen upon harvesting and stored at −80 °C. Samples were either packaged in aluminium foil in ten groups of ten krill (January 2014–September 2014), or in vacuum packaging in two groups of ten krill and one bulk bag of ~80 krill (December 2014–September 2016). All samples were labelled with the date of their collection and bar-coded, linking them to their geographical location at the time of their collection. The krill were then freighted on dry ice to Hobart, Tasmania, then stored at −80 °C for later analysis.

The length of all krill was measured using 'Standard 1' from Kirkwood (1982), sexed using a dissecting microscope and weighed (wet mass) prior to analysis. Dry mass was calculated by multiplying the wet mass by 0.2278 (Virtue et al., 1993a). Only adult or sub-adult krill (30 – 60 mm in length) were used in the analyses presented here. All juvenile krill remained frozen for later analysis. The maturity stages of krill were obtained for 2015 and 2016 samples only, therefore krill were categorised as "male" or "female" and maturity stages were not used for interannual comparisons. Once sexed, three females and three males were analysed in two-week periods ($N = 6$), where period 1 started on 1 January 2014 and ended 15 January 2014. All subsequent periods followed on from this date. The variance observed within a two-week period was examined by sampling the total lipid content of 10 random krill (five from each sex) from within the same period and undertaking a one-way ANOVA for power analysis. This process indicated that three individuals of each sex produced a representative sample from within that period, giving the statistical power required for analysis.

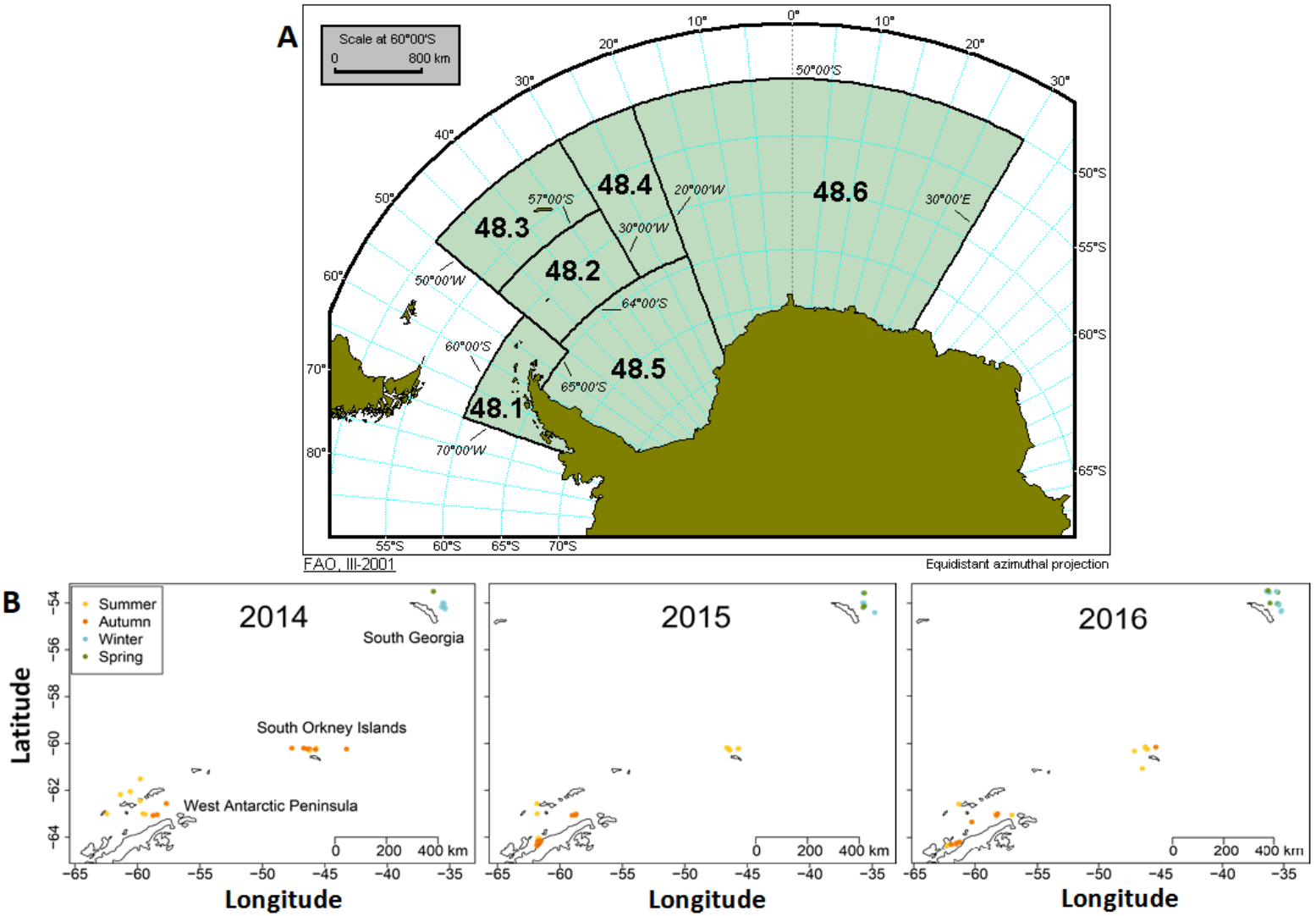

Figure 2.1. Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR) Statistical Area 48 and its Sub-Areas (from http://www.fao.org/fishery/area/Area48/en accessed 12/2/16) (**A**). Locations of collections of *Euphausia superba* by FV *Saga Sea* (Aker BioMarine) from January 2014 to September 2016 (**B**).

Lipid-extraction technique

Whole krill samples were quantitatively extracted overnight using a modified Bligh and Dyer (1959) method consisting of a methanol:dichloromethane:water (MeOH:CH_2Cl_2:H_2O) solvent mixture (20:10:7 ml). Phase separation was achieved the next day by adding 10 ml CH_2Cl_2 and 10 ml Milli-Q H_2O (saline), giving a final methanol:dichloromethane:water solvent ratio of 1:1:0.85. The lower layer was drained and the total lipid (TL) was concentrated using rotary evaporation. The TL was transferred into a pre-weighed 2 ml vial and the solvent was blown down under nitrogen gas to quantify total lipid content mass (mg). Solvent was added until further procedures were carried out to avoid oxidation.

The lipid class composition of samples was determined of the krill total lipid content using an Iatroscan MK-5 TLC/FID analyser (Iatron Laboratories, Tokyo, Japan). A standard lipid class solution sourced from Sigma; with known quantities of wax esters (WE), triacylglycerols (TAG), free fatty acids (FFA), sterols (ST), and phospholipids (PL), was used to calibrate the flame ionisation detector, with hydrocarbon (HC) (squalene) also used in a separate solution. Hydrocarbons (HC) and WE and steryl esters (SE) were combined for all statistical analyses (HC refers to this combination hereon), as these were only minor peaks and not all samples contained all of these lipid classes.

Aliquots (1µl) of the total lipid extract were spotted on chromarods and developed in accordance to Sutton (2015), with slight modifications for krill lipid classes such as a solvent system of hexane:diethyl-ether:acetic acid (90:10:0.1 ml) and a drying time of 5 min at 50 °C. Total lipid content per gram of krill mass is expressed as mg g^{-1} and abbreviated as TL DM (milligrams of total lipid extract per gram of krill dry mass).

Statistical analyses

The R statistical package (R Core Team, 2017) used for analysis was R version 3.4.2 (2017 - 09-28). The platform x86_64-w64-mingw32/x64 (64 bit) was utilised for all analyses. The R

packages used to undertake the statistics and create the figures were reshape2 (Wickham, 2007), ggplot2 (Wickham, 2009), gapminder (Bryan, 2015), effects (Fox, 2003), gplots (Warnes, 2016), ggpmisc (Aphalo, 2016), devtools (Wickham, 2017), knitr (Xie, 2018), maps (Becker, 2017), pwr (Champely, 2018), and lattice (Sarkar, 2008).

Total lipid, TL DM, and lipid class quantitative and percentage data for each season were analysed in the RStudio statistics package (version 0.99.893) using a multifactorial ANOVA, with sexual maturity and year as factors. Type 3 SS analyses were used to check statistical outputs for data levels that were unbalanced, but did not significantly alter output results, so Type 1 SS were used. To identify significant differences between factor levels, Tukey post-hoc comparisons were used. Log or square root transformations were utilised when data did not meet assumptions of normality. Location was not included as a factor as the fishing locations differed between seasons and years. Data in tables are expressed as mean ± standard deviation. For all analyses, α was set at 0.05.

RESULTS

Krill length and mass

The mean body length (Standard length 1; Kirkwood (1982)) of krill was 46.0 mm (± 4.8) and the mean dry mass was 0.16 g (± 0.05). Krill were longest in autumn (47.43 mm ± 3.76 mm) and were generally lighter and shorter during winter (0.264 g ± 0.09 g and 44.73 mm ± 4.26 mm) and heavier in summer (0.362 g ± 0.12 g). Krill sampled in the winter of 2015 were heavier and longer than krill in the previous or subsequent winters (p: 0.03; Table 2.1). Krill length and mass followed a seasonal trend ($p < 0.001$; Table 2.1). Krill mass and length significantly correlated with krill total lipid content ($r^2 = 0.87$ and 0.93, respectively, $p < 0.001$; Table 2.1).

Table 2.1: Average total lipid (mg g^{-1} dry mass, mean ± SD), length (mm) and mass (g) of *Euphausia superba* by sex, season and year. Seasons are defined as summer (1 December to 28 February), autumn (1 March to 31 May), winter (1 June to 31 August), and spring (1 September to 30 November).

	Total lipid content (mg g^{-1} dry mass)		**Average length (mm)**	**Average dry mass (g)**
	Males (N = 190)	Females (N = 201)		
Summer 2014	148.9 ± 116.2	148.0 ± 58.7	48.19	0.21
Autumn 2014	329.8 ± 68.3	268.6 ± 101.3	47.23	0.17
Winter 2014	210.6 ± 86.2	203.8 ± 66.4	42.04	0.11
Spring 2014	59.8 ± 12.7	114.8 ± 30.0	45.66	0.14
Summer 2015	168.1 ± 130.1	166.7 ± 102.1	46.49	0.17
Autumn 2015	309.2 ± 60.8	303.8 ± 74.2	45.63	0.14
Winter 2015	234.1 ± 60.7	233.5 ± 64.3	48.42	0.17
Spring 2015	138.9 ± 17.2	131.3 ± 16.5	48.95	0.20
Summer 2016	271.8 ± 128.3	217.2 ± 94.3	46.77	0.19
Autumn 2016	399.7 ± 51.8	361.3 ± 96.6	48.51	0.21
Winter 2016	209.0 ± 46.1	208.0 ± 57.2	44.01	0.13
Spring 2016	90.7 ± 26.6	120.1 ± 9.3	42.97	0.13

Total lipid content

Total lipid content varied significantly between seasons ($p < 0.001$; Fig. 2), years (p: 0.006; Fig. 2), and between sexes (p: 0.039; Table 2.1), but these variations were not evident in the sex and season interaction (p: 0.167; Table 2.1). A clear seasonal trend in lipid content of krill was seen across all years (Fig. 2.2), with the highest lipid content measured during autumn (508 mg g^{-1} TL DM) and the lowest during summer (41 mg g^{-1} TL DM). Total krill lipid content followed the same trend across all three years, increasing from summer to an autumn high, before declining throughout winter into spring lows. Total lipid in samples from each season were distinctly different from each other, except for summer and winter as total lipid levels in these two seasons were similar though they were increasing in summer and decreasing in winter (p: 0.113). The absolute levels in total lipid for each season differed between years (Table 2.2). The season and year interaction had a significant effect on the total lipid content ($p < 0.001$; Fig. 2.2), but this was driven mostly by the differences between 2014 and 2016 (p: 0.004). The differences between 2014 and 2016 were driven by their summer and autumn TL DM values being so dissimilar ($p < 0.0001$ for both); similarly, total lipid in autumn 2016 was significantly higher than in autumn 2015 (p: 0.018; Table 2.1).

Table 2.2: Lipid content and lipid class composition in *Euphausia superba* expressed by year and season (mean). Seasons are defined as: summer (1 December to 28 February), autumn (1 March to 31 May), winter (1 June to 31 August) and spring (1 September to 30 November). HC, hydrocarbons (includes steryl esters and wax esters present in trace amounts); TAG, triacylglycerols; FFA, free fatty acids; ST, sterols; DAG, diacylglycerols; PL, phospholipids.

		Total lipid	**Total lipid**	**Lipid class composition (%)**						
	N	**mass (mg)**	**(mg g^{-1} dry mass)**	HC	TAG	FFA	ST	DAG	PL	Unknown
Summer 2014	33	33.6	148.4	1.4	33.2	9.7	4.7	0.9	48.9	0.1
Autumn 2014	45	54.1	300.6	0.7	45.9	2.8	2.4	1.2	47.0	0.1
Winter 2014	44	23.6	201.9	1.3	35.6	1.4	1.8	0.6	59.2	0.0
Spring 2014	6	12.1	87.3	0.8	28.1	1.9	2.4	0.4	66.3	0.0
Summer 2015	30	30.9	183.9	0.9	31.2	1.4	2.2	1.0	62.6	0.7
Autumn 2015	42	44.9	315.1	1.6	42.1	0.1	2.7	2.8	50.5	0.0
Winter 2015	38	42.9	233.8	1.1	40.9	0.2	2.3	0.7	54.6	0.0
Spring 2015	12	27.4	135.1	1.1	34.9	0.8	2.5	0.7	59.9	0.0
Summer 2016	44	45.5	243.6	0.6	39.1	2.5	2.2	1.5	53.9	0.3
Autumn 2016	37	82.6	379.9	0.4	46.6	0.5	2.7	2.9	46.7	0.2
Winter 2016	38	27.4	208.5	0.5	41.9	0.7	1.5	0.9	54.4	0.1
Spring 2016	12	14.0	105.4	0.5	27.6	1.2	1.6	0.5	68.5	0.0

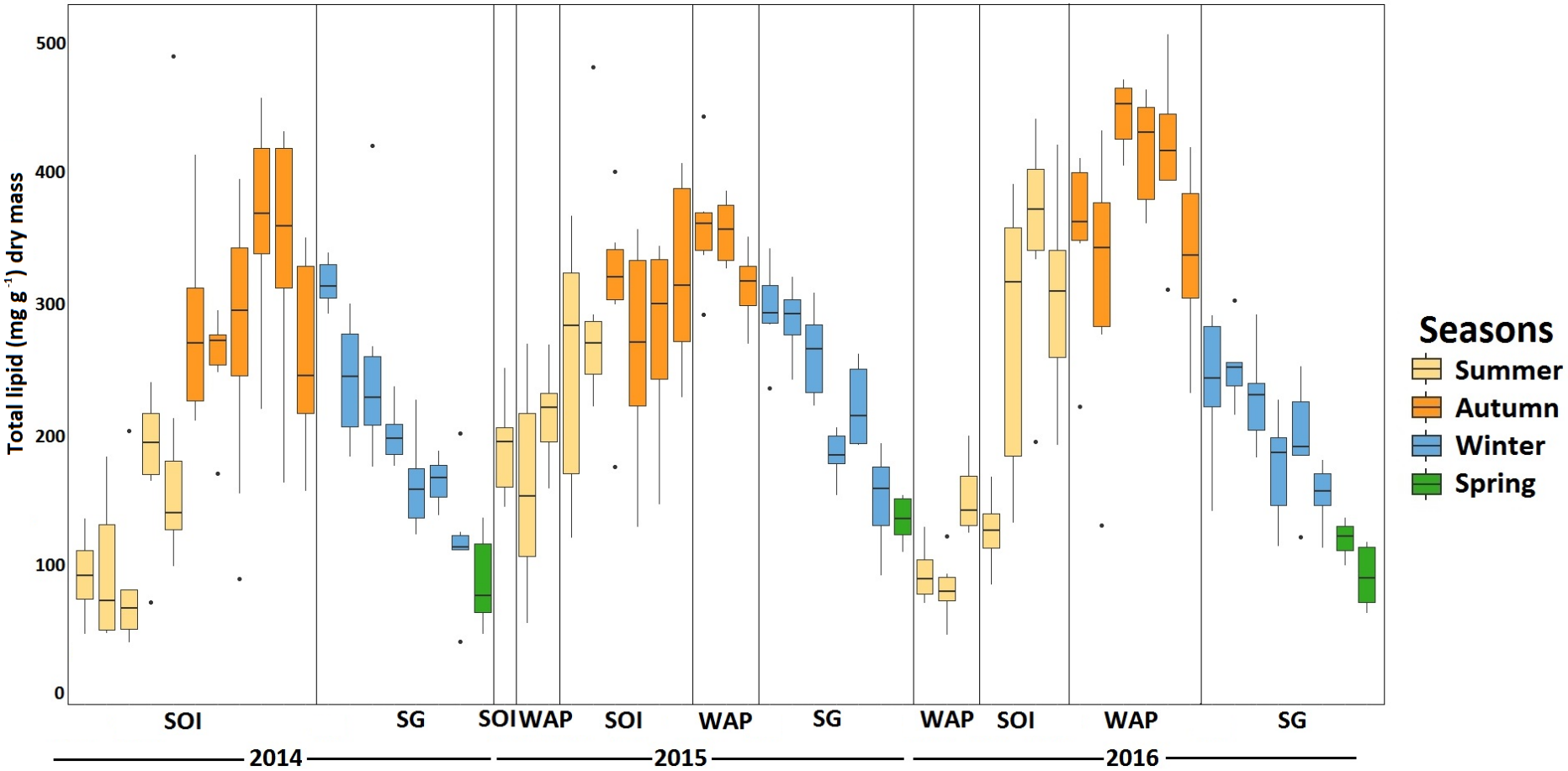

Figure 2.2. Total lipid content (mg g^{-1} of dry mass) of *Euphausia superba* showing the year and location of sample collection. The boxes from left to right show the two-week period of sample collection (where period 1 is January 1 to 15, 2014). Each box is the combination of three male and three female *Euphausia superba* from that period (N = 6). Each box represents 1 SD, with the whiskers the second SD and the bold line the mean. WAP, West Antarctic Peninsula; SOI, South Orkney Islands; SG, South Georgia.

The differences seen between sexes in their TL DM were variable over time (Table 2.1). All krill sampled in summer showed similar values for TL DM, except 2016, where males had higher TL DM than females (Table 2.1). Males exhibited higher average TL DM than females in autumn 2014 and 2016; however, no difference between sexes was seen in autumn 2015 (p: 0.155; Table 2.1). Winter krill showed no difference between the sexes (p: 0.999). Spring krill exhibited more variability, with females in 2014 and 2016 showing higher average TL DM than males, although no difference between the sexes was seen in spring 2015 (Table 2.1). This pattern of difference between the sexes was significant (p: 0.004), but the Sex*Season interaction (p: 0.167), Sex*Year interaction (p: 0.966), and Sex*Season*Year interactions (p: 0.704) were not. The analyses of female and male krill were therefore combined for all subsequent analyses on total lipid content as they had exhibited the same interannual and seasonal patterns in their total lipid content.

Lipid class composition

Lipid class percentages (expressed as % of TL) varied between seasons and years. There were significant differences in TAG levels between years (p: 0.028), seasons (28% in spring 2016 to 47% in autumn 2016; $p < 0.001$) and sex (p: 0.01). A significant Year*Season interaction (p: 0.002; Table 2.2) as well as a strong Sex*Year effect ($p < 0.001$) and Sex*Season*Year interaction ($P = 0.005$; Table 2.1) could be seen. Spent female krill (having spawned their eggs) had different levels of TAG compared to other female maturity stages and males (p: 0.02; Table 2.3).

Table 2.3: Lipid composition of *Euphausia superba* by sex and maturity stage for 2015–2016 samples (%, mean ± SD). HC, hydrocarbons (includes steryl esters and wax esters in trace amounts); TAG, triacylglycerols; FFA, free fatty acids; ST, sterols; DAG, diacylglycerols; PL, phospholipids; 3M, mature males; 3F, mature females; 3F-G, gravid females; 3F-S, spent females; 2F, sub-adult females; 2M, sub-adult males.

		Total lipid (mg	**Lipid class composition (%)**						
Sex	**N**	**g^{-1} dry mass)**	HC	TAG	FFA	ST	DAG	PL	Unknown
3M	62	239.9 ± 128.1	0.5 ± 0.3	37.9 ± 11.8	1.4 ± 2.9	1.4 ± 1.7	1.7 ± 4.2	56.1 ± 11.5	0.2 ± 0.4
3F	37	216.3 ± 100.5	0.5 ± 0.4	37.2 ± 9.0	1.7 ± 3.1	1.8 ± 0.9	0.9 ± 0.7	57.5 ± 9.1	0.1 ± 0.2
3F-G	5	113.3 ± 39.4	0.3 ± 0.1	25.1 ± 18.8	1.8 ± 1.5	2.6 ± 0.9	0.8 ± 0.8	68.1 ± 16.4	1.1 ± 1.0
3F-S	4	96.7 ± 43.0	1.0 ± 0.6	19.7 ± 18.6	2.6 ± 2.3	2.9 ± 1.4	0.7 ± 0.5	70.3 ± 13.4	2.1 ± 2.3
2F	94	254.5 ± 100.1	0.6 ± 0.4	40.6 ± 7.3	0.8 ± 1.2	1.9 ± 2.0	1.1 ± 1.0	54.6 ± 7.1	0.2 ± 0.5
2M	51	257.4 ± 106.0	0.5 ± 0.4	38.9 ± 9.5	1.3 ± 1.5	1.8 ± 1.3	1.1 ± 1.2	55.9 ± 8.2	0.3 ± 1.1

The dominant lipid class for all krill samples was PL (58 ± 7.4%), which had the widest range of values between samples (23 to 88%; Table 2.2). Levels of PL in krill varied significantly with sex (*p*: 0.018; Table 2.3) and season (*p*: 0.041) and showed strong Year*Sex effects (*p*: 0.025; Table 2.3). Levels of PL in the summer and spring of 2015 were significantly different to those in 2014 and 2016 ($p < 0.001$; Table 2.2), whereas PL levels in all other seasons and years were not ($p > 0.05$ for all). PL levels in gravid females and spent females were not significantly different, but PL levels in both gravid and spent females differed from sub-adults and other mature krill of both sexes (Table 2.3).

When all seasons were combined, there was a relationship between PL and TAG ($y = -0.835x + 86.955$, where y is the phospholipid percentage and x is the triacylglycerol percentage). This relationship is much stronger and has a better fit in the winter ($r^2 = 0.956$) and spring ($r^2 = 0.94$) than in the summer ($r^2 = 0.618$) and autumn ($r^2 = 0.651$) (Fig. 2.3). The greater variation in this relationship in the summer was driven by 2014 samples, which had a much greater spread of values.

FFA levels also did not significantly differ between years and seasons. Male krill had a significantly lower FFA level than all female stages except spent females ($p < 0.001$). FFA had large Year*Sex and Season*Sex interactions ($p < 0.001$ for both sexes; Table 2.3). FFA levels also exhibited a significant Year*Sex*Season interaction (*p*: 0.013; Tables 2.2 and 2.3), with differences between sexes and all maturity stages particularly in 2014. Sterol (ST) levels also showed a significant sex effect ($p < 0.001$). Whilst levels of diacylglycerol (DAG) in krill were fairly consistent (Table 2.2), there were a few outliers early in 2014 and one two-week period of significantly higher DAG levels in 2016 which were driven by higher levels in mature male krill.

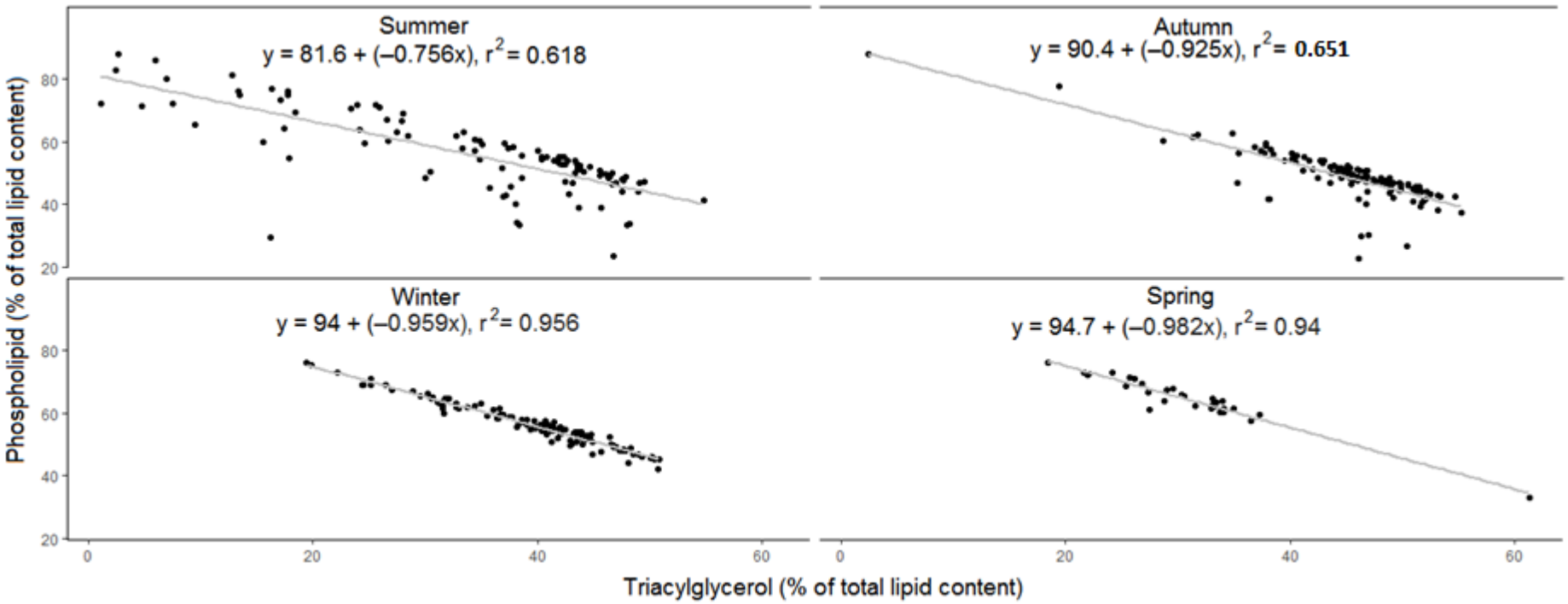

Figure 2.3. The seasonal relationships between phospholipids and triacylglycerol (as % of the total lipid content) from fortnightly samples of *Euphausia superba* collected from January 2014 to September 2016.

The levels of hydrocarbons (HC) were significantly different between years, seasons and sexes ($p < 0.001$ for all) when tested independently by ANOVA. When analysed using a multifactorial ANOVA and passed through Type III SS and F tests, however, there were no significant differences or interactions ($p > 0.1$ for all; Tables 2.2 and 2.3). No larger pattern is evident even if a Tukey test showed some significant differences between individual seasons or years or sexes.

Quantitative lipid class analysis

The absolute mass of TAG (mg) in individual krill varied seasonally and interannually (Fig. 2.4A), with TAG content being more variable in the summer and autumn. The variations align more closely to the seasonal and interannual trend seen in the lipid class composition data when TAG was corrected for by krill mass (and expressed as mg g^{-1} DM) (Fig. 2.4B). FFA content increased slightly in samples collected at the beginning of 2016. ST content was highly stable across all three years and between seasons, whether looking at absolute ST mass, or the ST mass scaled to krill mass or total lipid content. PL showed the same seasonal and interannual trend as in the percentage data when looking at the absolute mass of PL (Fig. 2.4C). The anomalous highs in autumn 2016 are captured in this trend, but not when PL are standardised against krill dry mass (Fig. 2.4D).

The seasonal trend seen in TAG content (Fig. 2.4A) was muted significantly when TAG content was scaled to total lipid content (Fig. 2.5A). Such a result shows that the amount of total lipid content in the krill affects the quantity of TAG content more than krill size (g) does. Although the same seasonal trend is seen in PL when data are scaled to krill mass, the trend seen in PL all but disappears once the amount of total lipid content is used for scaling (Figs. 2.4D and 2.5B). No discernible trend in the PL content as a proportion of total lipid content could be detected, although this may be due to the high levels of variability throughout 2014 and early 2015 (Fig. 2.5B).

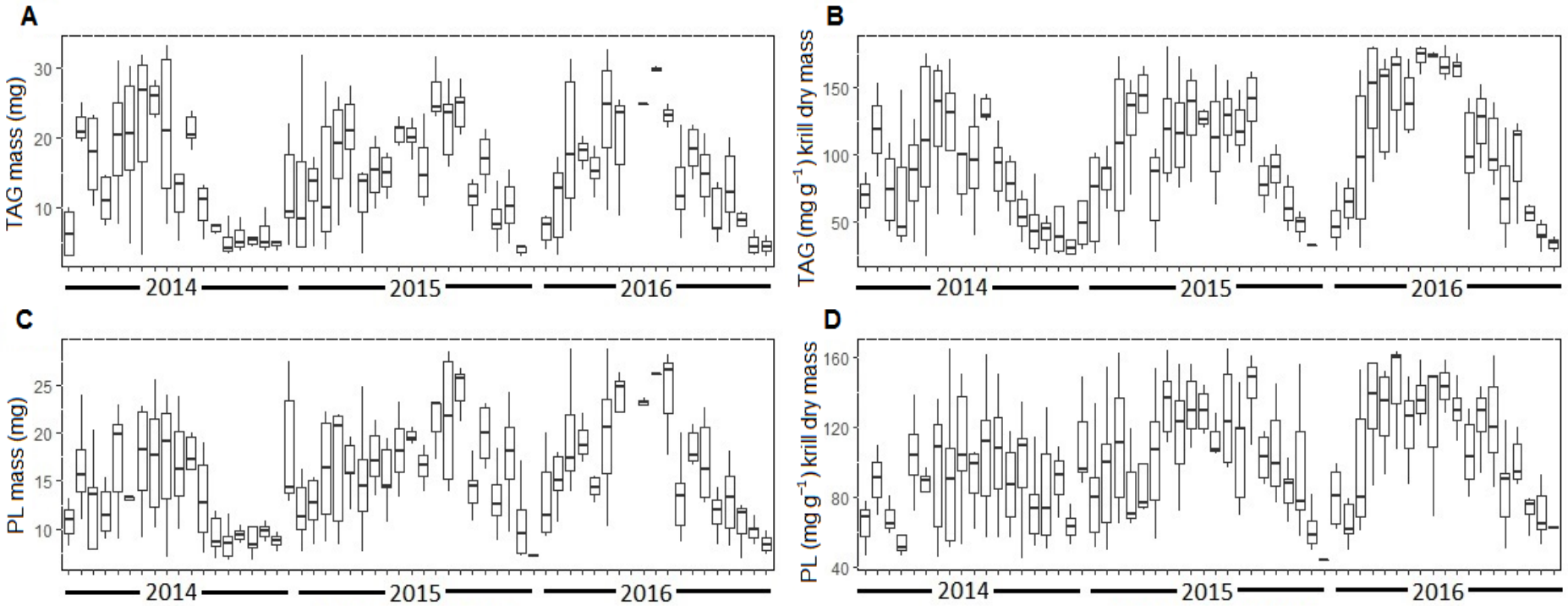

Figure 2.4. Quantitative trends in the major lipid classes during 2014 to 2016; TAG mass (mg per krill) (**A**). TAG of krill dry mass (mg g^{-1}) (**B**). PL mass (mg per krill) (**C**). PL of krill dry mass (mg g^{-1}) (**D**). Each box is the combination of three male and three female *Euphausia superba* collected from a two-week period (N = 6). The first period of sample collection was January 1–15, 2014. Each box represents 1 SD, with the whiskers the second SD and the bold line the mean. TAG, triacylglycerols; PL, phospholipids.

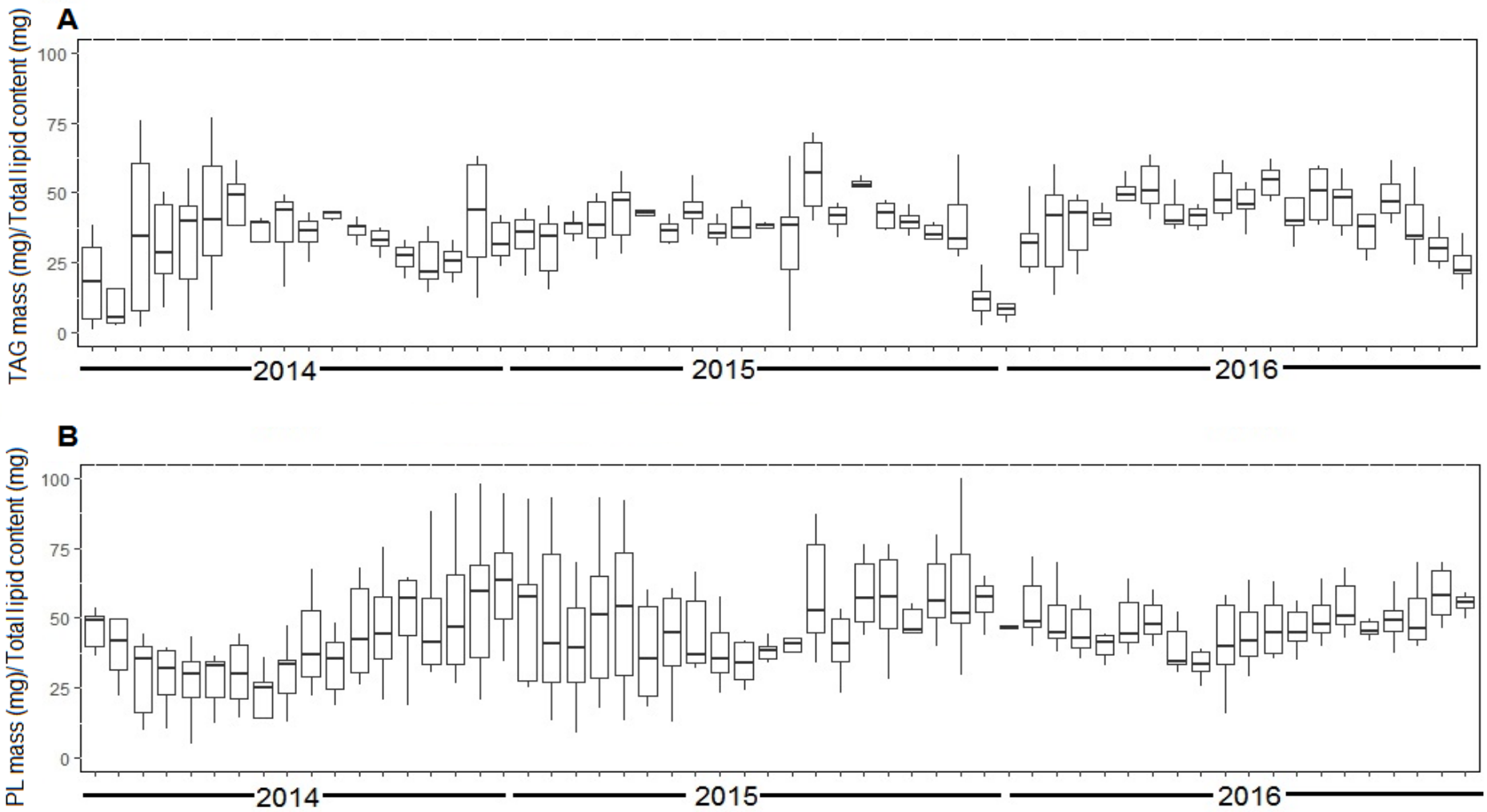

Figure 2.5. Triacylglycerol (TAG) mass (mg) as a proportion of the total lipid mass (mg) of *Euphausia superba* (**A**). Phospholipids (PL) mass (mg) as a proportion of the total lipid mass (mg) of *Euphausia superba* (**B**). Each box represents 1 SD, with the whiskers the second SD and the bold line the mean.

DISCUSSION

Our study has resolved the sinusoidal shape of the seasonal trend in krill lipid content and composition by utilising high-resolution fisheries-derived samples. Krill lipids exhibit a clear seasonal trend in their total content and in their component lipid classes. The seasonal differences observed in the major lipid classes reflect their differing roles in key physiological and biochemical processes: growth, storage, and reproduction. These seasonal cycles have become clearer through the use of high-resolution sampling.

Effect of sex on the lipid seasonal cycle in krill

The lipid content and the relative proportions of lipid classes in krill differed significantly between seasons. These seasonal trends have similarly been seen at a coarser resolution in Antarctic krill (Clarke, 1984, Fricke et al., 1984, Quetin et al., 1994, Phleger et al., 2002, Hagen and Kattner, 2014). Pond et al. (1995) and Ju and Harvey (2004) reported that lipid accumulation is tightly linked to seasonal factors such as the timing of reproduction in krill. Such differences were also shown in the northern krill *Meganyctiphanes norvegica* (Sars, 1857) by Falk-Petersen (1981) and Cuzin-roudy et al. (1999), who reported that the timing of lipid accumulation in relation to reproduction was vital.

The sex of krill had little effect on the amount of total lipid regardless of year or season. This result differs from the results of previous studies which reported that gravid female krill have higher total lipid content due to the development of the ovary prior to spawning in the late summer or early autumn (Virtue et al., 1996, Cuzin-roudy et al., 1999). Males at this time of year also had elevated total lipids (Table 2.1), indicating that extra energy stores may be required during the mating process as Virtue et al. (1996) suggested. The lower total lipid values in females compared to males during the spawning season might be due to females losing large quantities of lipid when they spawn (Table 2.3). Females, however, have higher lipid stores in spring leading into the spawning season (Table 2.1).

The lipid content and lipid class composition of reproductive krill is well studied (Clarke, 1984, Pond et al., 1995, Virtue et al., 1996, Mayzaud et al., 1998, Atkinson et al., 2002) but the differences between the sexes and how differing lipid content and composition impacts on their reproduction is not well understood. Mayzaud et al. (1998) reported that sub-adults and females had a similar wet weight to TAG content relationship, whereas males showed a particularly different relationship. Age-dependent use of lipid stores has been reported at the onset of winter, with adult krill having higher lipid stores than sub-adults but similar lipid class compositions (Atkinson et al., 2002). Female krill from South Georgia had higher lipid levels on average and were longer and heavier than male and sub-adult krill in summer (Pond et al., 1995). Tarling et al. (2016a) reported that growth and shrinkage in krill across the South Atlantic was sex-dependent. This suggests that mature males and females in the spawning seasons should have sex-dependent growth, although this may not necessarily match their sex-dependent lipid content and composition.

Effect of krill length and mass on the seasonal lipid cycle

Total lipid content varied with krill mass and length, with bigger krill having higher overall lipid content than smaller ones (as reported by Falk-Petersen et al. (2000) and Gigliotti et al. (2011)). We found that the longest krill were not always the heaviest. Krill in summer were heavier than the longest krill in autumn except in 2016. This may be due to many krill in summer being gravid females heavy with eggs. Post-spawn females were larger but lighter than gravid females due to the loss of egg mass. If lipid contents in krill are proportional to size, as well as maturity stage in females, this could be significant for recruitment and energy flow in the krill life cycle.

Krill were significantly smaller and leaner in the summer of 2014; these krill also generally showed higher PL and lower TAG levels (Table 2.2). These low TAG levels could be due to the summer of 2014 being exceptionally warm (0.57 °C warmer than average for the

global oceans; NOAA (2013) and Wiedermann et al. (2016)), causing food supplies to be lower and diatoms to be less abundant (Ericson et al., 2018a). A change from a predominantly diatom diet to a dinoflagellate diet in *M. norvegica* halted ovary development due to the reabsorption of lipids from ovaries, which was mostly TAG (Cuzin-Roudy et al., 2004). Significantly lower levels of TAG could be a sign of poor krill health and therefore recruitment in the summer of 2014 as TAG, along with PL, is one of the major energy sources in krill.

Triacyclglycerol and phospholipid seasonal cycles in krill

The seasonal levels of TAG followed the sinusoidal trend seen in total lipid content with a peak in autumn. PL also had a seasonal response but with the peak occurring in spring (Table 2.2). The relationship between PL and TAG was highly variable in all summer samples. This variability may be due to differences in the timing and spatial extent of algal blooms after the spring melt (Skerratt et al., 1995, Janout et al., 2016). Such effects have been suggested for northern krill (Falk-Petersen, 1981). Gravid krill have much higher levels of TAG than spent females because krill eggs are high in TAG. Observed TAG levels in female krill would thus be dependent on the timing of krill spawning.

TAG, however, is a storage lipid and would be expected to fluctuate according to the energetic needs of the krill and the availability of food throughout the year (Hagen, 1996, Atkinson et al., 2002). Krill increase their TAG levels throughout late spring and summer when food is abundant, causing TAG levels to rise and be at their highest in autumn (Hellessey et al., 2018). Krill use these stored lipids over winter and hence TAG levels are at their lowest at the end of spring when food starts to become more abundant again (Korb et al., 2005, Vernet et al., 2008, Schmidt et al., 2012, Kohlbach et al., 2018). Food supplies increase throughout spring and summer, and krill have a higher energy demand to fuel reproduction and growth during this time of year (Kawaguchi, 2016), so storing lipids at this same time is minimal. TAG levels have the same sinusoidal pattern seen in total lipid content and underlie the changes in total

lipid content at the seasonal scale. PL levels, however, showed the reverse seasonal relationship to TAG. A decrease in PL levels in autumn is accompanied by a rise in TAG from summer levels. When krill are at their smallest (shortest and lightest) during winter, their PL levels are low, whilst their total lipid content and TAG levels are still high. Similarly, krill are large in summer, and their PL levels are high, whilst their TAG levels and total lipid contents are low.

Phospholipids are vital for healthy cell membranes and as krill grow and accumulate more cells, their PL levels would increase proportionally. Because of the ability of krill to shrink and re-grow to full size (Ikeda and Dixon, 1982, Tarling et al., 2016a), there are likely to be fluctuations in their PL levels accompanying their growth and shrinkage. The length and mass of krill were significantly correlated with PL levels and these inversely followed the peaks and troughs of the seasonal cycle of their lipid content (Tables 2.1 and 2.2). PL may be conserved preferentially as it is used in the structure of krill cell membranes. Krill in this study had inconsistent PL content throughout the year and it was not conserved preferentially. PL has been suggested as a storage fat (Hagen et al., 1996, Daly, 2004, Ju and Harvey, 2004) and the inconsistent content of PL seems to suggest the same. PL levels are also important in the production of krill oil rich in PL-containing omega-3 fatty acids.

There was one exception to the proportional seasonal relationship, between the content of PL and TAG. Krill had disproportionally low TAG levels in summer. Greater increases in TAG levels from the spring could be driving this pattern, but further sampling throughout the spring would be required to clarify this.

Sterol content showed little seasonal trends and was highly consistent whether in terms of its total mass, or when scaled to krill mass or total lipid content (Table 2.2). This observation suggests that sterols may not be used as much as previously thought for the mobilisation of krill lipids, which is why these levels are consistent throughout the krill life cycle and across years and seasons.

Our results also show a clear seasonal trend and some interannual variation in adult krill lipid profiles. TAG content followed the same seasonal pattern as TLDM and could be shown to behave as a storage lipid alongside PL in winter in adult krill. The seasonal trends of lipid content and composition in juvenile krill are not well understood with limited available data regarding interannual trends (Atkinson et al., 2002, O'Brien et al., 2011, Virtue et al., 2016, Schaafsma et al., 2017). Future analyses of fishery-derived samples may assist with understanding lipid trends in juvenile krill.

Our study also indicates the utility of samples collected by the krill fishery. Other studies have utilised data from such samples, but these have not been at a high level of temporal resolution (Kim, 2014, Tarling et al., 2016b). Fisheries samples are not ideally collected for ecological studies and because our samples were collected across the Scotia Sea, and in different seasons, our results may be confounded to a degree. Separating the spatial and temporal elements from the lipid data will require more samples from multiple locations at the same time of year, or year-round sampling from a single location (e.g. South Georgia, which is accessible year-round). Alternatively, a combination of sampling by multiple fishing vessels and by scientific research vessels may ensure complete seasonal or regional coverage.

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Chapter 3: Regional variability of Antarctic krill (*Euphausia superba*) diet during the late-summer as determined using lipid, fatty acid and sterol composition

This Chapter is currently under review at Polar Biology (submitted 18th March 2019).

ABSTRACT

Antarctic krill (*Euphausia superba*) are a circumpolar species with an omnivorous diet. Knowledge of krill diet in different oceanic regions will help predict how regional-scale environmental change may impact local krill populations. Krill from the Atlantic, Indian and Pacific sectors of the Southern Ocean were compared. The total lipid, lipid class, and neutral fraction fatty acid and sterol content and composition of whole krill, their digestive glands and stomachs during the late-summer were examined. Krill from the Indian sector had a distinctly different diet to the Atlantic and Pacific sectors based on their fatty acid profiles (p: < 0.001). Indian sector whole krill had higher phospholipids (55.0 ± 8.9%, as % total lipids) compared to Pacific (45.9 ± 3.6%) and Atlantic sector krill (43.7 ± 8.2%). Indian sector krill digestive glands showed lower phospholipid levels (Indian: 29.4 ± 8.5%, Pacific: 52.5 ± 5.7%, Atlantic: 52.5 ± 5.9%). Indian sector krill had a more carnivorous and diatomaceous diet (higher levels of 16:1n-7c, 14:0 and 20:1 and 22:1 isomers), with less flagellate input (lower 18:4n-3, 21:5n-3 and 18:3n-6) than other regions. One site in the Indian sector had particularly high 22:6n-3. Indian Ocean sector krill had lower cholesterol levels in their stomachs (52.5 ± 14.1%, as % total sterols) than Pacific and Atlantic sector krill stomachs (62.8 ± 1.9 % and 60.9 ± 4.9%, respectively). This study is, to our knowledge, the first to detail the regional differences in late-

summer krill diet by assessing the lipid, neutral fraction fatty acid and sterol content and composition of different tissue types.

INTRODUCTION

Antarctic krill (*Euphausia superba*, hereon krill) are a keystone species in the Antarctic ecosystem (Murphy et al., 2007, Barnes and Tarling, 2017). Krill are extremely lipid rich (up to 40% of dry mass in winter (Hagen et al., 2001, Atkinson et al., 2002)), and are therefore very energy dense, making them an ideal food source for other animals in the harsh Antarctic environment. Krill have a circumpolar distribution and live in a variety of environments (Atkinson et al., 2008, Atkinson et al., 2009, Jarvis et al., 2010, Kawaguchi et al., 2010b, Leonori et al., 2017). Krill lipid profiles have been used to detect their health, condition and diet (Schaafsma et al., 2017, Ericson et al., 2018a, Hellessey et al., 2018). It is unclear how lipid profiles vary with shorter term dietary and environmental changes.

Krill diet varies seasonally and regionally (Ericson et al., 2018a) and with maturity stage (Virtue et al., 1997), although these observations generally have been based on analyses of the lipid profiles from whole krill. Such observations may not reflect the shorter term diet of the krill which can be better observed in measurements from individual body parts such as the digestive gland and stomach (Mayzaud et al., 1998, Schmidt and Atkinson, 2016, Schaafsma et al., 2017).

The fatty acid profile in the neutral lipid fraction, containing mostly the storage lipid triacylglycerol, reflects the diet to a greater degree than fatty acids in phosphoplipids or total lipids. As triacylglycerol is accumulated during feeding, the use of neutral lipid fatty acid profiles of digestive and stomach tissue, rather than whole animal tissue, allows for even greater understanding of dietary components. To date only a few studies have been conducted using fatty acid markers from the neutral lipid fraction, however, these studies used whole krill tissue (Pond et al., 1995, Ju and Harvey, 2004, Schaafsma et al., 2017). Only one study by

Cabrol et al. (2019) has looked at the fatty acids in neutral lipid fractions of different tissue types in Northern Atlantic krill to determine diet. No studies to date, however, have investigated the neutral lipid fraction in different krill tissue types for Antarctic krill.

Krill accumulate lipids in the austral summer during the spawning season and these reach their highest concentrations in autumn (Hellessey et al., 2018). Krill are known to have divergent lipid class stores and fatty acid profiles depending on their sex (Mayzaud et al., 1998) and particularly if they are gravid or are post-spawn females (Mayzaud et al., 1998, Hellessey et al., 2018). Krill lipid profiles are also a function of their diet, however, differences in krill diet at a circumpolar scale at this time of year are generally unknown. The lipids in krill are utilised by both the megafauna that prey on krill (Mori and Butterworth, 2004, Trivelpiece et al., 2011) and the commercial krill fishery (Nicol et al., 2012, Kwantes and Grundmann, 2015). Although predation by vertebrates is highest in the summer months, the krill fishery is most active in autumn and winter (Nicol et al., 2012).

Currently the Antarctic krill fishery is largely based in the Atlantic sector of the Southern Ocean despite being almost circumpolar in nature when it first came into operation in the 1970's (Hill et al., 2016, CCAMLR, 2017). There has been little recent krill fishing in the Pacific and Indian sectors of the Southern Ocean (Nicol and Foster, 2016, CCAMLR, 2017).

The South Atlantic, especially the West Antarctic Peninsula, is an area of extremely rapid environmental change and is the fastest warming region in the world (Ducklow et al., 2007), having experienced a midwinter surface air temperature warming of 5 – 6 °C in the last 50 years (Rayner, 2003, Ducklow et al., 2007). An associated 40% reduction in sea ice duration, extent and concentration has been recorded over the last 26 years (Ducklow et al. (2007)). This has led to a community level shift in the lower trophic levels (Deppeler and Davidson, 2017, Hancock et al., 2018), particularly in the primary producers which krill feed upon. This

reduction of sea ice and its associated communities are likely to affect krill diet and population dynamics. Sea ice variability has been associated with krill recruitment and population stability (Brierley et al., 2002, Wiedenmann et al., 2009, Schmidt et al., 2014), because juvenile krill are thought to rely on sea ice algae during their first winter (Ross and Quetin, 2000). The combination of reduced sea ice and warming seas in this region may affect both the short- and long-term diet of krill and thus their health and ability to reproduce (Kawaguchi, 2016). Lipid analysis can be a useful tool to investigate any shift in krill diet and condition (Virtue et al., 2016, Schaafsma et al., 2017, Ericson et al., 2018a).

The Indian and Pacific sectors of the Southern Ocean have lower temperatures and more stable sea ice coverage than the South Atlantic sector (Nicol et al., 2000c, Ducklow et al., 2007, Barnes and Tarling, 2017). Both the Indian and Pacific sectors harbour large populations of krill, but these populations have not been as well studied as those in the southwest Atlantic.

Circumpolar krill studies use data from disparate time points (different months and seasons) or from large datasets using varying methodologies (e.g. KRILLBASE (Atkinson et al., 2017)) and these circumpolar datasets rarely have information on krill biochemistry or diet.

Understanding regional differences in krill diet will assist in identifying how krill cope with varying regional environmental conditions. Our aims were: 1) to investigate krill diet using neutral lipid fraction fatty acid and sterol content and composition of whole krill, 2) to use both krill stomach and digestive gland samples to investigate short term diet versus long term diet in whole krill samples and 3) to investigate differences in krill diet from samples collected in all three ocean basins surrounding Antarctica during the late-summer season. We hypothesise that krill from the three different oceanic basins around Antarctica will have vastly different diets at the crucial late-summer spawning period.

METHODS

Krill sample collection

We utilised krill from three sources. Krill were collected by Aker BioMarine on-board FV *Saga Sea* throughout February 2016 from the Atlantic sector of the Southern Ocean (CCAMLR Area 48 (Figure 3.1)) using a harvesting technology that continuously pumps krill from the cod-end of a submerged net (Hellessey et al., 2018). After capture, krill were individually stored on-board at −80 °C. Krill were collected on-board the RSV *Aurora Australis* throughout February 2016 as part of the K-Axis voyage from the Indian Ocean sector of the Southern Ocean (CCAMLR Area 58 (Figure 3.1)) using a Rectangular Midwater Trawl and stored individually on-board at −80 °C. Krill were collected on the RSV *Akademik Treshnikov* throughout February 2017 as part of the Antarctic Circumpolar Expedition in the Pacific Ocean sector of the Southern Ocean (CCAMLR Area 88 (Figure 3.1)) using a Bongo net. Krill were stored on-board at −20 °C, but showed no signs of lipid or fatty acid degradation (total free fatty acids: < 5% in the whole krill) due to this temperature storage difference. All krill samples were transported on dry ice (approx. −80 °C) to Hobart, Australia.

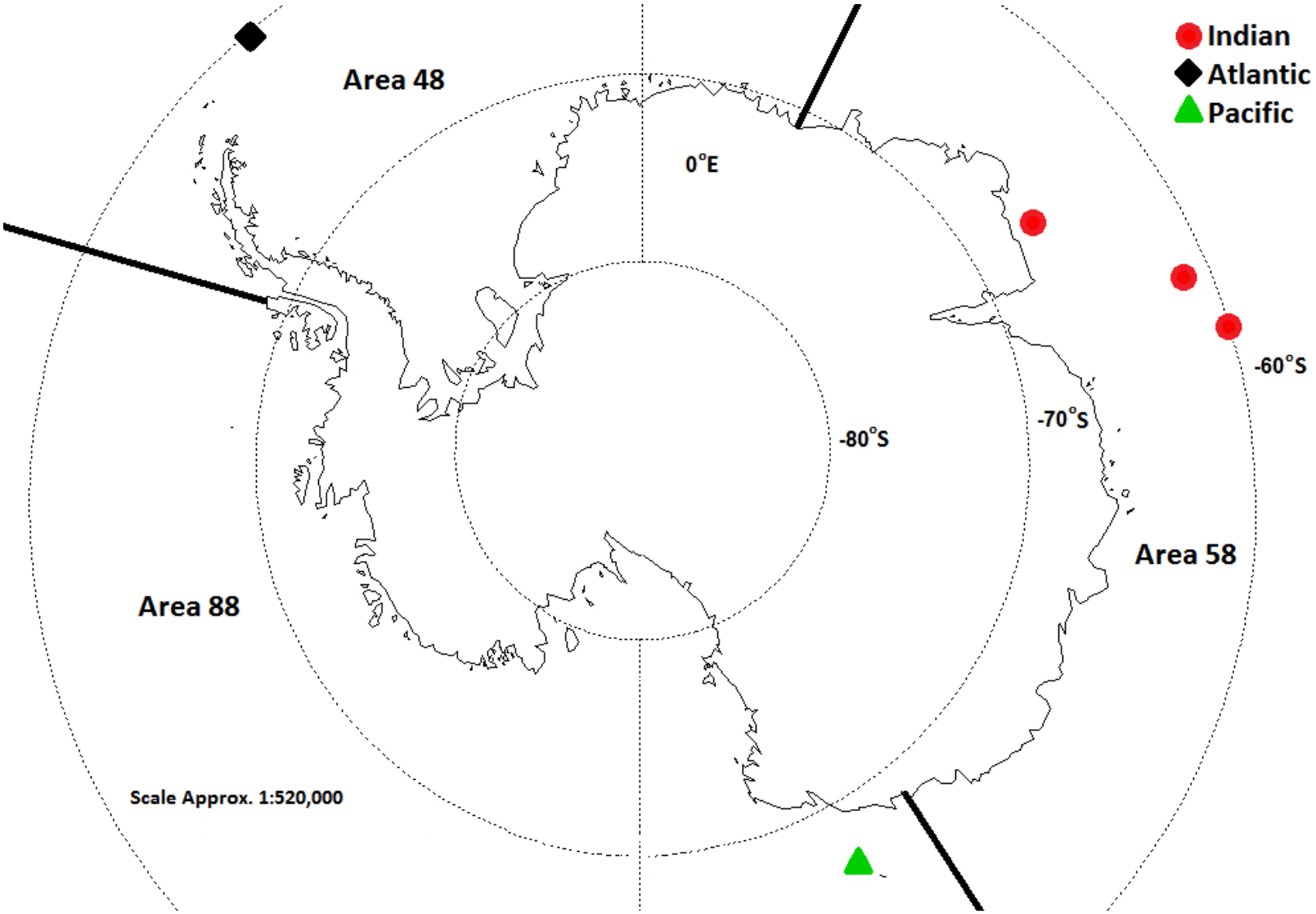

Figure 3.1: *Euphausia superba* sample collection locations coloured by their Southern Ocean basin and showing CCAMLR management areas.

Sample preparation

Individual krill were sized (Standard Length 1; Kirkwood (1982)), weighed and their maturity stage and sex were determined prior to analysis. Wet mass was converted to dry mass by multiplying by 0.2278 to account for the 77.2% water content in krill (Virtue et al., 1993a). Only adult or sub-adult krill were used in this study.

Lipids in whole krill reflect the long-term feeding history (Schmidt and Atkinson, 2016)); the digestive gland reflects shorter-term feeding history and the stomach lipids are reflective of what has been immediately eaten (Virtue et al., 1993a, Yoshida et al., 2009). Three gravid females, three spent females, three sub-adult females and three male krill were used for whole krill extractions (these whole krill samples included the digestive gland and stomach). The digestive glands were dissected from additional krill samples. Two digestive glands were used per sample to ensure a large enough lipid fraction at the end of the extraction process. Similarly, the stomachs were dissected out, weighed and combined (12 stomachs per sample) for extraction. All dissections took place in a glass petri dish sitting in an ice bath. Krill were consistently sexed, weighed and dissected in under 5 minutes to keep thawing to a minimum whilst samples were on ice.

Total lipid, fatty acid and lipid class extraction and analysis

Samples were quantitatively extracted overnight using a modified Bligh and Dyer (1959) method as described in Hellessey et al. (2018) to produce the total solvent extract (TSE). The total lipid content (TL, expressed as mg) of each sample was weighed gravimetrically in a pre-weighed 2 ml glass vial. To account for differences in krill size, the TL was divided by krill dry mass (g) and is expressed as mg of total lipid content per gram of krill dry mass (mg g^{-1}, TL DM). The TL DM was standardised by the number of krill per sample (whole krill = 1, digestive gland = 2, stomach = 12) to give the TL DM (mg g^{-1}) per krill. Lipid class composition was determined by analysis of the TSE on an Iatroscan TLC-FID analyser following Hellessey

et al. (2018). Briefly, an aliquot of the TSE was drawn up a 0.1 µl capillary tube and spotted onto glass chromarods and placed into a solvent bath (hexane (C_6H_{14}):diethyl ether (Et_2O): acetic acid (CH_3COOH), 90:10:0.1, v:v:v) for 25 minutes before being dried in an oven at 50 °C for 10 minutes. The chromarods were then run through an Iatroscan TLC-FID analyser (Parrish and Ackman, 1985). The identification and quantification of lipid classes (expressed as % total lipid content) was conducted in comparison to a known laboratory standard (sourced from Sigma) of wax esters (WE), triacylglycerols (TAG), free fatty acids (FFA), sterols (ST), and phospholipids (PL) and was used to calibrate the flame ionisation detector, with hydrocarbon (HC) (squalene) also used in a separate solution. Aliquots of sample TSEs were methylated to extract the fatty acid methyl esters (FAME) of the sample (Ericson et al. (2018a). In brief, an aliquot of the TSE was transferred to a glass test tube and methylated with 3 ml of solution (methanol (MeOH): dichloromethane (CH_2Cl_2): hydrochloric acid (HCl), 10:1:1, v:v:v). Samples were then heated to 90 – 100 °C for 75 minutes and cooled for 5 minutes before 1 ml H_2O and 1.8 ml (C_6H_{14}:CH_2Cl_2, 4:1, v:v) solution were added to extract FAME. Samples were centrifuged for 5 minutes and the upper layer transferred to a vial before an additional 1.8 ml of C_6H_{14}:CH_2Cl_2 was added. This process was carried out 3 times to ensure all FAME were extracted, and the vial was kept under nitrogen (N_2) gas between transfers. Sample vials were blown down to remove all solvents and were made up with 1 ml internal injection standard (23:0 FAME) (Iverson et al., 2004). Samples were analysed via gas chromatography (GC) using an Agilent Technologies 7890A GC-FID System (Palo Alto, CA, USA) equipped with a non-polar Equity.-1 fused-silica capillary column (15 m length x 0.1 mm internal diameter, 0.1 µm film thickness). Samples (0.2 µl) were injected in splitless mode at an oven temperature of 120 °C with helium as the carrier gas. The oven temperature was raised to 270 °C at a rate of 10 °C per minute, then to 310 °C at 5 °C per minute. Peaks were quantified using Agilent Technologies ChemStation software (Palo Alto, CA, USA) with initial identification based on

comparison of retention times with known (Nu Chek Prep mix; http://www.nu-chekprep.com) and fully characterised laboratory (tuna oil) standards. Fatty acid peaks were expressed as a percentage of the total fatty acid area.

Confirmation of component identification was performed by GC-MS of selected samples and was carried out on a Thermo Scientific (Waltham, MA, USA) 1310 GC coupled with a TSQ triple quadruple. Samples were injected using a Tripleplus RSH (Waltham, MA, USA) auto sampler using a non-polar HP-5 Ultra 2 bonded-phase column (50 m length x 0.32 mm internal diameter x 0.17 μm film thickness). The HP-5 column was of similar polarity to the column used for GC analyses. The initial oven temperature of 45 °C was held for 1 min, followed by temperature programming at 30 °C per minute to 140 °C, then at 3 °C per minute to 310 °C, where it was held for 12 min. Helium was used as the carrier gas. Mass-spectrometer operating conditions were as follows: electron impact energy 70 eV; emission current 250 μamp, transfer line 310 °C; source temperature 240 °C; scan rate 0.8 scan/sec and mass range 40–650 Da. Mass spectra were acquired and processed with Thermo Scientific XcaliburTM software (Waltham, MA, USA). Identification and quantification of peaks was conducted using the same standards as GC-FID analysis.

Column chromatography

Lipid class fractions of krill sample TSE were separated via column chromatography. The columns were packed with 1 g of activated silica and enough chloroform ($CHCl_3$) to just cover the silica. An aliquot of the lipid extract (approximately 10 mg) was syringed onto the top of the column. In some instances, due to samples having less than 10 mg of total lipid, the entire lipid extract was used. Lipid fractions were collected by eluting 20 ml of $CHCl_3$, then acetone (C_3H_6O) and finally MeOH, which were then each rotary evaporated to concentrate the individual lipid fractions. An Iatroscan TLC-FID analyser was used to confirm lipid class separation. All of the neutral lipid fractions from the digestive glands and stomachs were used

to prepare FAME of these samples using the same method as above. Neutral lipid FAME were analysed by GC-FID and GC-MS to identify and quantify fatty acid markers as described above.

Saponification

An aliquot of the neutral lipid fraction was treated with 2 ml saponifying solution (5% potassium hydroxide (KOH) in MeOH:MilliQ H_2O 80:20, v:v) and heated to 60 °C for 3 hours before being cooled and 1 ml MilliQ H_2O and 1.8 ml C_6H_{14}:CH_2Cl_2 added to extract the total non-saponifiable neutral (TSN) lipid. The TSN lipid fractions were then silyated with the addition of 50 µl N, O-bis (trimethylsilyl) trifluroacetamide and heated at 60 °C overnight. Prior to GC-FID analysis, samples were blown down under N_2 gas and 1 ml of internal injection standard (23:0 FAME) added. Silyated TSN samples were analysed by GC-FID and GC-MS similarly to the FAME samples to identify and quantify sterol markers.

Statistical analyses

All statistical analyses were conducted in RStudio (version 1.0.153 Copyright (C) 2016) using the packages: nlme (Pinheiro et al., 2017), maps (Becker, 2017), effects (Fox, 2003), ggplot2 (Wickham, 2009), multcomp (Hothorn et al., 2008), multcompView (Graves et al., 2015), lsmeans (Lenth, 2016). Due to the low number of males in the sample collections, sub-adult and mature males were pooled so that each site had three males for comparison to the three sub-adult, three gravid and three spent females from each site. Linear mixed effect models including a random factor, least squares comparisons and multifactorial ANOVAs were used to see which variables of Sector (Atlantic, Indian, Pacific), Site (2 in Atlantic, 3 in Indian, 1 in Pacific), Type (whole, stomach or digestive gland) and Sex (gravid female, spent female, sub-adult female and male krill) and their interaction terms were significant to the TL DM per krill content, as well as the individual lipid classes, and the neutral lipid fraction fatty acids and sterol levels and their associated masses. Nested ANOVAs were undertaken to look at differences in the sites from the same sector and the sexes from the same site and sector for all

other variables. Year was not included as a factor despite the Indian and Atlantic sectors being sampled in February 2016, whilst the Pacific was sampled in February 2017. Some confounding of year/site is to therefore be expected. Data were log or square root transformed when the assumptions of normality and homogeneity of variances weren't met.

Principal component analyses (PCA) were performed on the major fatty acids (> 0.5% of the total fatty acid profile) in Primer6 (version 6.1.13 Copyright (C) 2009) using Bray-Curtis similarity matrices. All data were pre-treated with a log transformation (logx+1) prior to PCA analyses.

RESULTS

Total lipid content

Krill from the Indian sector were generally shorter than krill from the other sectors, however, they were not significantly different in weight in any of their sample types (Table 3.1). Atlantic sector samples had higher TL DM (mg g^{-1}) per krill in all tissue sample types compared to Indian sector samples ($p < 0.006$). No significant differences in TL DM (mg g^{-1}) per krill were observed between samples from the Indian and Pacific sectors (p: 0.904) and there were only small differences between the Pacific and Atlantic sectors (p: 0.092; Figure 3.2). There was a strong tissue type effect and sector effect, although no significant interaction (Type*Sector) effect ($p < 0.001$, 0.04 and 0.80 respectively; Figure 3.2).

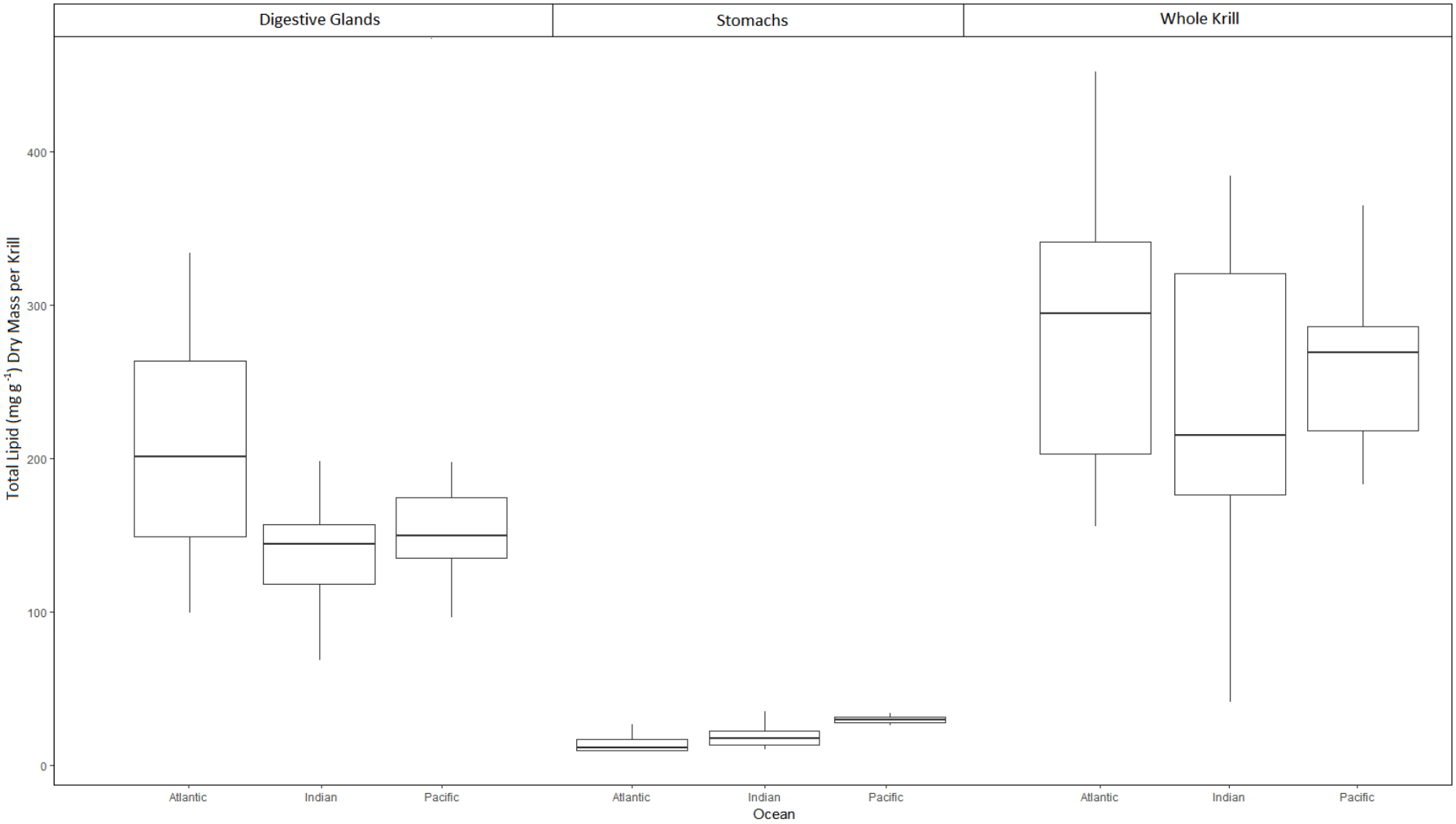

Figure 3.2: Total lipid content (mg g^{-1}) dry mass of *Euphausia superba* in different Southern Ocean sectors (Atlantic, Pacific and Indian) and tissue types (stomach, digestive gland and whole krill) per animal. Each box represents 1 SD, with the whiskers the second SD and the bold line the mean.

Table 3.1: Length (mm), wet weight (g) and total lipid content (mean ± SD) of different *Euphausia superba* tissue sample types from the Atlantic, Indian and Pacific Southern Ocean sectors. TL: Total lipid, DM: dry mass. N = number of samples (1 krill per whole krill sample, 2 krill digestive glands per sample and 12 krill stomachs per sample).

Sample Type	Sector	N	Length (mm)	Wet Weight (g)	TL (mg)	TL (mg g^{-1}) DM
Whole Krill	Atlantic	18	50.1 ± 3.2	0.94 ± 0.18	66.3 ± 28.5	306 ± 89.2
	Indian	24	46.4 ± 5.5	0.83 ± 0.32	44.3 ± 17.0	257 ± 97.8
	Pacific	12	45.1 ± 4.3	0.80 ± 0.29	46.3 ± 14.3	263 ± 56.7
Stomach	Atlantic	4	51.8 ± 6.9	0.01 ± 0.00	5.51 ± 2.94	254 ± 97.0
	Indian	6	46.7 ± 5.2	0.01 ± 0.01	7.36 ± 7.24	232 ± 108
	Pacific	2	52.5 ±7.8	0.01 ± 0.00	11.1 ± 4.83	300 ± 63.1
Digestive gland	Atlantic	12	50.8 ± 2.4	0.08 ± 0.02	14.9 ± 7.76	414 ± 48.2
	Indian	18	44.5 ± 6.8	0.07 ± 0.03	8.24 ± 4.11	279 ± 73.0
	Pacific	6	53.6 ± 5.5	0.15 ± 0.06	20.8 ± 11.2	304 ± 67.6

Sex had a small but insignificant overall effect on the TL DM per krill of samples (*p*: 0.053). There were no significant differences between individual sex classes and the small differences seen were mainly due to males having higher TL DM per krill (304.4 ± 89.3 mg g^{-1}) than spent females (236.1 ± 98.2 mg g^{-1}) (*p*: 0.07). Tukey tests revealed that Sector had a more significant impact on TL DM per krill content than the sex of the sample did ($p > 0.1$ for all sex classes).

Lipid class composition

Digestive gland, stomach and whole krill lipid class compositions were not significantly different from each other (*p*: 0.25, Figure 3.3). Most differences in lipid class composition were driven by regional differences, with the Indian sector being significantly different to the Atlantic and Pacific sector samples ($p < 0.001$ for both, Figure 3.3). Atlantic and Pacific sector samples were not significantly different to each other (*p*: 0.22). There was, however, a strong Type*Sector interaction for overall lipid class composition (*p*: 0.002; Figure 3.3).

Both the mass (mg) and percentage (%) levels of FFA (0.0 ± 0.1 mg and 2.9 ± 5.6%), ST (ST: 0.0 ± 0.0 mg and 0.8 ± 3.6%), DAG (0.0 ± 0.0%) and PL (0.7 ± 0.6 mg and 29.4 ± 8.5%) were lower in the Indian sector digestive gland samples than the Atlantic and Pacific sector samples (Table 3.2 and Supplementary Table 3.1 (Appendix 1)). Pacific sector digestive glands had the highest FFA and PL mass and percentages, followed by Atlantic sector samples, with Indian sector digestive glands having the lowest mass and percentages of FFA and PL (Tables 3.2 and Supp. Table 3.1). Pacific digestive gland samples had the lowest HC (includes wax and steryl esters) mass and percentages (0.01 ± 0.01 mg and 0.2 ± 0.3%) and the lowest TAG percentages (33.7 ± 3.9%) of all the digestive gland samples (Atlantic: 36.3 ± 6.6%, Indian: 57.9 ± 19.1%).

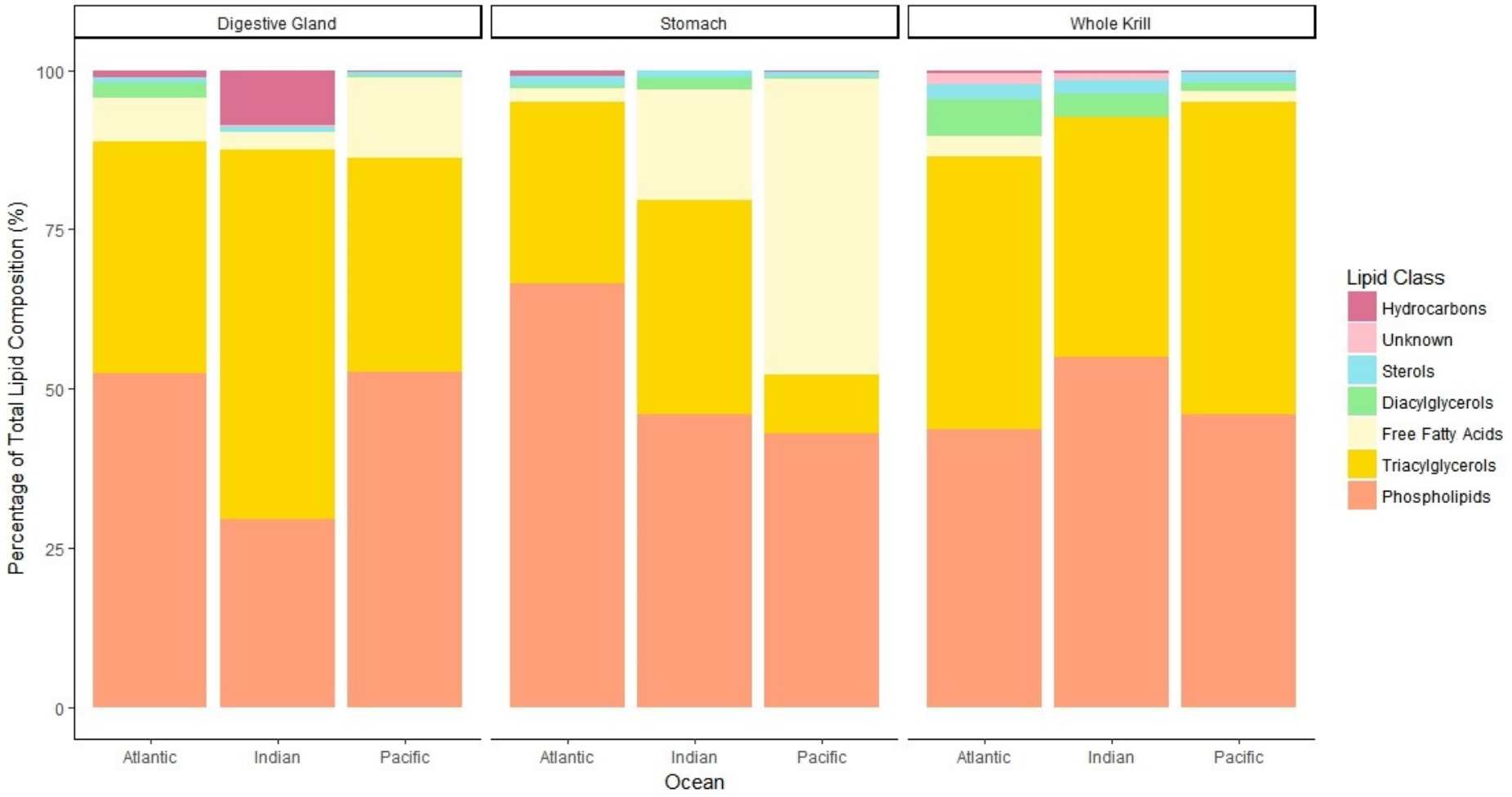

Figure 3.3: Lipid class composition (% of total lipids) between Southern Ocean sectors (Atlantic, Pacific and Indian) and tissue types (stomach, digestive gland and whole krill) of *Euphausia superba* samples. Hydrocarbons includes wax and steryl esters.

Table 3.2: Mass (mg g^{-1}, mean ± SD) of each lipid class for each *Euphausia superba* sample, tissue types (digestive gland, stomach and whole krill) and Southern Ocean sectors (Atlantic, Indian and Pacific). Mass for the stomach and digestive gland samples are for the whole sample (2 digestive glands per sample and 12 stomachs per sample) and are not on a per krill basis. HC - hydrocarbons (including wax and sterol esters); TAG - triacylglycerols; FFA - free fatty acids; ST - sterols; PL - phospholipids.

Sample Type	Sector	HC	TAG	FFA	ST	PL
Whole Krill	Atlantic	0.4 ± 0.6	18.7 ± 11.2	1.7 ± 0.7	1.3 ± 1.9	17.0 ± 13.0
	Indian	0.1 ± 0.2	12.0 ± 6.5	0.1 ± 0.0	0.5 ± 0.6	13.8 ± 5.7
	Pacific	0.1 ± 0.0	16.7 ± 5.6	0.6 ± 1.2	0.5 ± 0.3	13.7 ± 4.7
Stomach	Atlantic	0.1 ± 0.0	1.0 ± 0.7	0.1 ± 0.0	0.0 ± 0.0	1.8 ± 1.1
	Indian	0.0 ± 0.0	0.8 ± 0.7	0.5 ± 1.1	0.4 ± 0.8	1.5 ± 1.7
	Pacific	0.1 ± 0.0	0.6 ± 0.5	4.2 ± 3.3	0.0 ± 0.0	2.3 ± 0.3
Digestive gland	Atlantic	0.0 ± 0.0	3.3 ± 2.5	0.7 ± 0.3	0.1 ± 0.1	3.7 ± 2.2
	Indian	0.2 ± 0.3	1.4 ± 0.7	0.0 ± 0.0	0.0 ± 0.1	0.7 ± 0.6
	Pacific	0.0 ± 0.0	3.8 ± 2.8	1.5 ± 1.1	0.0 ± 0.0	5.0 ± 3.7

Pacific sector stomach samples had the lowest PL mass (2.3 ± 0.3 mg) and percentages (43.0 ± 16.4%), as well as the lowest TAG mass (0.6 ± 0.5 mg) and DAG percentages (0.2 ± 0.3%) (Tables 3.2 and Supp. Table 3.1). Indian sector krill stomachs had low HC and FFA mass (0.00 ± 0.01 mg and 0.5 ± 1.1 mg, respectively) and HC percentages (0.00 ± 0.02%) compared to other stomach samples, whilst Atlantic sector stomachs had low ST mass and FFA percentages (Tables 3.2 and Supp. Table 3.1).

Indian sector whole krill samples followed the same trend as their digestive glands with low HC and TAG mass (0.1 ± 0.2 mg and 12.0 ± 6.5 mg, respectively) and low TAG and FFA percentages (37.6 ± 10.7% and 0.1 ± 0.1%, respectively). Atlantic sector whole krill samples had the highest mass and percentages for most lipid classes (Tables 3.2 and Supp. Table 3.1).

There were no significant differences in lipid class levels between sexes, except for PL (*p*: 0.036). This difference was driven by spent females in the Indian sector which were significantly higher in their percentage of PL (66.5 ± 13.8%) than spent females in the other regions (Atlantic: 41.6 ± 9.5%, Pacific: 46.6 ± 5.7%). Indian sector krill were generally higher in PL levels than observed for the other regions except in gravid females which were consistent in their PL levels regardless of the region.

Quantitatively, TAG mass varied slightly but insignificantly with sex class (*p*: 0.053), due to differences between males and sub-adult females (18.3 ± 10.4 mg and 9.9 ± 4.2 mg, respectively) (*p*: 0.06). There was also a large difference in PL mass depending on sex class (*p*: 0.01), with sub-adult females and males (5.5 ± 1.9 mg and 18.7 ± 12.3 mg, respectively) (*p*: 0.042) having the largest difference in PL mass, but sexes within the same region didn't show a significant difference (*p*: 0.68).

Fatty acid content and composition

The sum of fatty acid groups (monounsaturated (MUFA), polyunsaturated (PUFA) and saturated fatty acids (SFA)) did not vary significantly between tissue types or regions (Table 3.3). Some fatty acids were combined or used as ratios to look at particular dietary indicators such as: carnivory (18:1n-9c/18:1n-7c), copepod consumption (Σ 20:1n-9c+22:1n-9c, Hagen et al., 1995), marine snow and bacterial indicators (Σ C_{15}, C_{17} and C_{19} isomers, hereon termed MSI), diatom consumption (Σ 16:4n-1+16:1n-7c), and the copepod to diatom ratio (Σ 20:1n-9c+22:1n-9c/16:4n-1+16:1n-7c) for an estimate of their herbivory: omnivory: carnivory ratio.

The biggest differences in the fatty acids of the total lipid between tissue types were seen in the major fatty acids 20:5n-3 (EPA), 22:6n-3 (DHA), 18:4n-3 (SDA), MSI, copepod, carnivory and diatom markers, phytol (side chain of chlorophyll, used as a primary production marker) and the copepod to diatom ratio (Tables 3.3 and Supplementary Table 3.2 (Appendix 1)). Indian sector samples had the lowest fatty acid mass (MSI, copepod and diatom markers) and relative levels (MSI and carnivory markers) in their total lipids overall, although not in their 16:0, 16:1n-7c, copepod biomarker percentages and copepod to diatom ratio levels (Tables 3.3 and Supp. Table 3.2). 16:4n-1 and EPA and copepod marker levels were consistently highest in Atlantic sector digestive glands and whole krill, followed by Pacific sector samples, with the lowest relative levels in Indian sector samples (Tables 3.3 and Supp. Table 3.2). Similarly, DHA, SDA, MSI and carnivory marker levels were highest in Pacific sector samples, followed by the Atlantic sector and were lowest in Indian sector samples, regardless of sample type (Tables 3.3 and Supp. Table 3.2).

Table 3.3: *Euphausia superba* fatty acid groups (expressed as mg g^{-1} sample; mean ± SD) and selected major dietary fatty acid markers in different sectors of the Southern Ocean (Atlantic, Indian and Pacific) and tissue types (whole krill, stomach and digestive glands). MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, SFA: saturated fatty acids, MSI: marine snow indicator [Σ C_{15}, C_{17} and C_{19} isomers], Copepods: [Σ 20:1n-9c + 22:1n-9c], Diatoms: [Σ 16:1n-7c +16:4n-1].

Sample Type	Sector	MUFA	PUFA	SFA	MSI	Phytol	Copepods	Diatoms
Whole Krill	Atlantic	13.2 ± 5.6	16.3 ± 4.9	16.1 ± 6.6	0.3 ± 0.1	1.3 ± 0.9	1.0 ± 0.6	4.2 ± 1.7
	Indian	6.5 ± 5.3	6.8 ± 4.1	8.2 ± 8.1	0.1 ± 0.5	0.8 ± 0.4	0.3 ± 0.2	2.2 ± 2.1
	Pacific	11.1 ± 2.5	15.1 ± 3.5	14.6 ± 4.2	0.4 ± 0.1	0.8 ± 0.3	0.6 ± 0.2	2.7 ± 0.8
Stomach	Atlantic	17.3 ± 19.5	16.9 ± 16.4	16.8 ± 14.8	0.3 ± 0.2	0.5 ± 0.7	1.0 ± 1.0	4.2 ± 3.8
	Indian	10.4 ± 7.0	11.5 ± 7.8	11.1 ± 5.8	0.2 ± 0.2	0.0 ± 0.0	0.5 ± 0.3	2.9 ± 1.4
	Pacific	12.2 ± 4.8	17.7 ± 6.8	13.9 ± 5.6	0.4 ± 0.2	0.4 ± 0.6	0.8 ± 0.2	2.6 ± 0.9
Digestive gland	Atlantic	15.8 ± 5.1	19.9 ± 4.8	20.3 ± 6.9	0.4 ± 0.1	1.1 ± 0.5	1.3 ± 0.4	5.0 ± 1.7
	Indian	9.2 ± 3.5	10.2 ± 3.0	13.0 ± 5.8	0.2 ± 0.1	0.0 ± 0.0	0.5 ± 0.2	3.7 ± 1.9
	Pacific	12.9 ± 2.8	17.6 ± 3.6	16.1 ± 4.1	0.4 ± 0.0	0.8 ± 0.2	0.8 ± 0.2	3.1 ± 1.0

The differences seen between whole krill from the different regions are clearly established in a PCA plot of the total lipid fatty acids (Figure 3.4A), which shows the distinctly different Indian sector krill. This difference was mostly driven by higher levels of 16:1n-7c, 14:0 and 20:1n-9c and lower levels of 18:4n-3 and DHA in Indian sector krill compared with krill from the other regions (cumulative percentage variation: 86.8%). The fatty acids having the biggest influence were SDA, DHA and 16:1n-7c (Figure 3.4A, see Supplementary Table 3.3 (Appendix 1) for PCA eigenvalues). PC1 was driven by 16:1n-7c and SDA, PC2 was driven by DHA and 14:0 and PC3 was driven by 14:0 and 20:1n-9c (Figure 3.4A and Supp. Table 3.3). Other fatty acids made up smaller parts of each component, but were not the driving factors of the differences seen (e.g. EPA, 18:1n-7c, 20:4n-3 and 16:4n-1) (Figure 3.4A and Supp. Table 3.3).

The differences in regional dietary influences are more clearly seen using stomach and digestive gland neutral lipid fraction fatty acids (Figures 3.4B and 3.4C, respectively). Digestive glands showed a smaller difference between regions, with more overlap observed between their neutral lipid fraction fatty acid profiles (Figure 3.4C) than the distinctly different regional neutral lipid fraction fatty acid profiles observed in the stomach samples (Figure 3.4B). The regional differences seen in the stomach neutral lipid fraction fatty acid profiles are driven by 14:0, 18:0, DHA and 16:1n-7c (cumulative percentage variation: 95.3%, see Supplementary Table 3.3 (Appendix 1) for full PCA values). The differences between regions in digestive gland profiles are driven by 14:0, EPA, 18:1n-9c and SDA (cumulative percentage variation 92.4%, see Supp. Table 3.3 for full PCA values). SDA made up a significant but smaller portion of the variation in the stomach PCA (Figure 3.4B and Supp. Table 3.3).

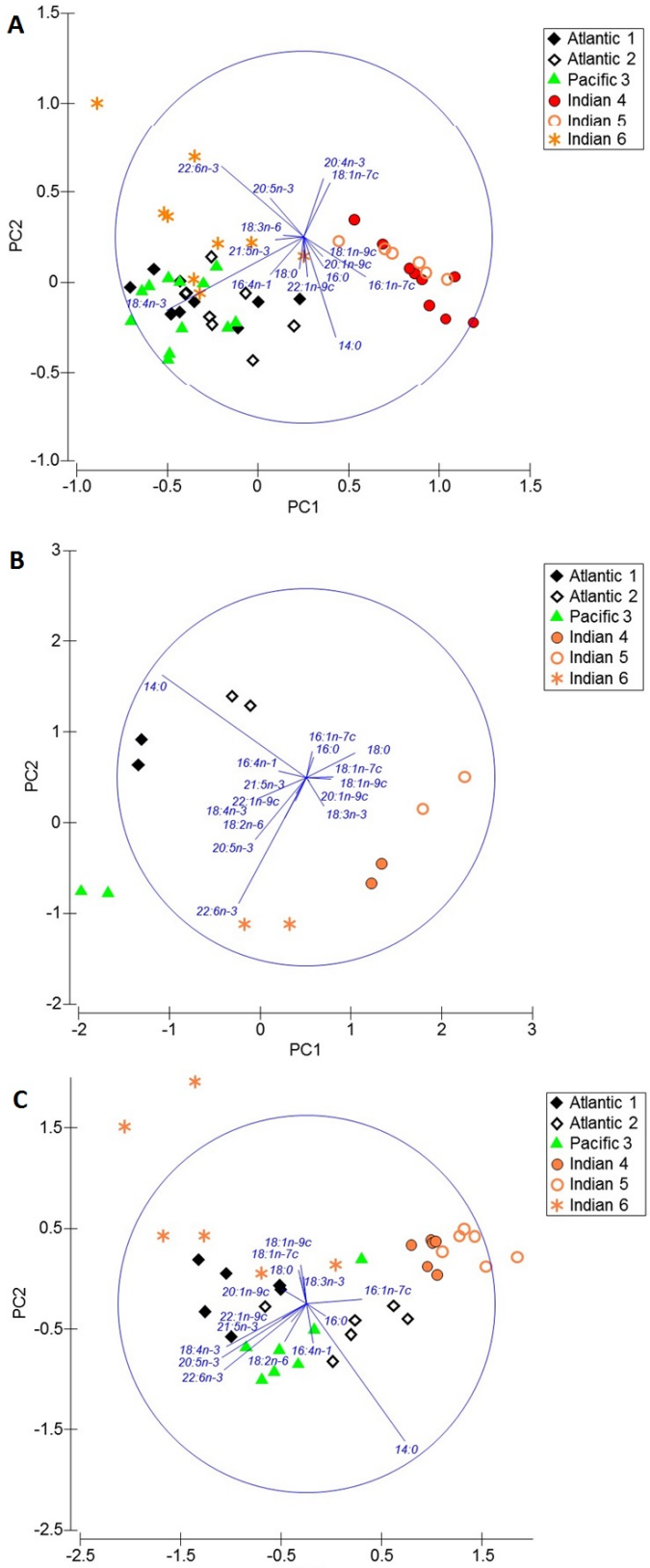

Figure 3.4: Principal Component Analysis of the fatty acid composition (% data) of *Euphausia superba* samples from different Southern Ocean sectors (Atlantic, Pacific and Indian) and sites from: (A) the total lipid of whole krill, (B) the neutral lipid fraction of krill stomachs and (C) neutral lipid fraction of krill digestive glands.

The different fatty acid groups (MUFA, PUFA and SFA) in the total lipid of whole krill varied slightly, but not significantly between sex classes. Again, the Indian sector krill were distinctly different in their fatty acid composition, and this was driven mostly by sub-adult females which were high in 16:1n-7c (Figure 3.5). The principle components that caused this separation are identical to those seen in Figure 3.4A (Supp. Table 3.1). However, a significant difference between the same sexes at different sites within the Indian sector could also be seen (Figures 3.4A and 3.5). Krill from the site closest to the continental shelf (Indian 6) were more similar to the krill of the Atlantic and Pacific sectors (higher DHA and SDA, lower 16:1n-7c), whereas krill from the more oceanic sites (Indian 4 and 5) had distinctly different dietary markers. This continental shelf site had no sub-adult females present, which was the driving sex class in the other Indian sector sites.

Fatty acid masses were quantitatively lower in all sexes of Indian sector krill. Atlantic sector males had more 16:0 (12.7 ± 5.5 mg, mean ± SD), 16:1n-7c (4.8 ± 2.2 mg), 16:4n-1 (0.3 ± 0.1 mg), EPA (9.8 ± 3.7 mg), and copepod marker (1.3 ± 0.8 mg) masses than Pacific sector males (9.5 ± 3.9 mg, 2.5 ± 1.2 mg, 0.2 ± 0.1 mg, 7.5 ± 2.3 mg and 0.7 ± 0.3 mg, respectively). Pacific sector sub-adult females had higher fatty acid masses than Indian sector sub-adult females, regardless of fatty acid type or ratio, except in their 16:1n-7c mass which was lower (Pacific: 3.4 ± 0.5 mg, Indian: 3.7 ± 3.9 mg). Spent females in the Atlantic sector were higher in all fatty acid masses except for their MSI and carnivory markers (0.4 ± 0.1 mg and 1.9 ± 0.2 mg, respectively), which were higher in spent females in the Pacific sector (0.5 ± 0.1 mg and 2.1 ± 0.2 mg, respectively). Gravid females in the Pacific sector were higher in DHA and SDA (4.2 ± 0.5 mg and 1.6 ± 0.2 mg), and in their MSI and carnivory marker (0.6 ± 0.1 mg and 2.2 ± 0.1 mg) masses than gravid females in the Atlantic (DHA: 4.0 ± 1.6 mg, SDA: 1.5 ± 0.5 mg, MSI: 0.5 ± 0.1 mg, Carnivory: 1.8 ± 0.2 mg) and Indian sectors (DHA: 1.7 ± 0.9 mg, SDA: 0.3

± 0.3 mg, MSI: 0.2 ± 0.1 mg, Carnivory: 1.7 ± 0.1 mg). Atlantic sector gravid females were higher in 16:0, 16:4n-1, EPA and copepod marker masses.

Sterols

Cholesterol levels did not differ between regions or sample types (*p*: 0.55 and 0.69) and there was no Type*Sector interaction (*p*: 0.79; Table 3.4). Pacific sector samples were generally higher in the major sterol percentages (cholesterol: 62.8 ± 1.89% (stomach) and 47.8 ± 11.24% (digestive gland), desmosterol: 26.5 ± 2.87% (stomach) and 28.0 ± 9.48% (digestive gland) and brassicasterol: 3.8 ± 0.39% (stomach) and 6.27 ± 1.79% (digestive gland)) on a mass basis than samples from the other regions, except for 24-methylenecholesterol in their digestive gland (2.0 ± 0.54%) and 24-ethylcholesterol in their stomach (0.1 ± 0.45%) samples being low (Table 3.4). Indian sector samples were lower in selected sterols except 24-ethylcholesterol levels which were higher in digestive gland (1.0 ± 0.58%) and stomach samples (0.8 ± 0.56%) than the other regions (Atlantic sector stomachs: 0.3 ± 0.11% and digestive glands: 0.4 ± 0.18%, Pacific sector stomachs: 0.1 ± 0.45% and digestive glands: 0.3 ± 0.06%.

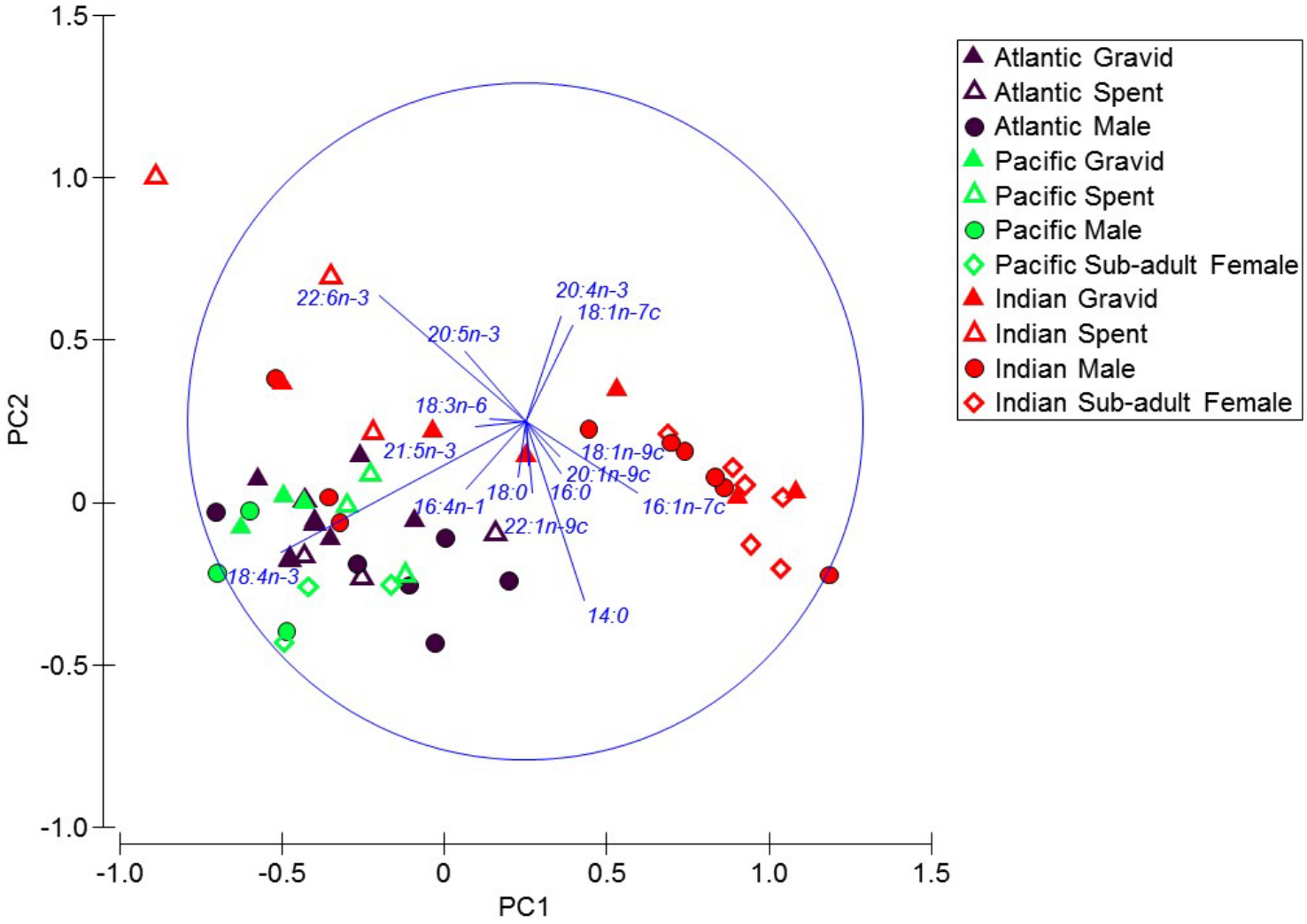

Figure 3.5: Principal Component Analysis of the total lipid fatty acid composition (% composition) compared by sex between Southern Ocean sectors (Atlantic, Pacific and Indian) of whole *Euphausia superba* samples.

Table 3.4: Sterols of *Euphausia superba* digestive gland and stomach samples by Southern Ocean sector (Atlantic, Indian and Pacific) expressed as a percentage (%, mean ± SD) of the total sterol profile.

Sample Type	Sector	N	24-Norcholesta-5,22E-dien-3ß-ol (24-Norcholesterol)	Cholesta-5,22Z- dien-3ß–ol (Trans-22-dehydrocholesterol)	Cholest-5-ene-3 ß –ol (Cholesterol)	24-Methyl-cholesta-5,24-diene-3ß–ol (Desmosterol)	24-Methyl-cholesta-5,22E-diene-3ß–ol (Brassicasterol)	24-(Methylenecholesterol) 5,24(28)E-diene-3ß–ol	24-Methyl-cholesta-	24-Ethyl-cholest-5-ene-3ß–ol 24-(Ethylcholesterol)	Σ Unknown
Stomach	**Atlantic**	4	1.01 ± 0.22	2.48 ± 0.40	60.90 ± 4.93	28.65 ± 6.61	2.30 ± 0.87	2.72 ± 0.87	0.31 ± 0.10	1.56 ± 0.17	
	Indian	6	0.95 ± 0.73	7.38 ± 5.55	52.50 ± 14.10	18.31 ± 9.56	3.52 ± 2.18	1.74 ± 0.93	0.81 ± 0.56	14.81 ± 6.43	
	Pacific	2	0.91 ± 0.19	4.40 ± 0.53	62.81 ± 1.89	26.51 ± 2.87	3.80 ± 0.69	1.08 ± 0.45	0.14 ± 0.45	0.37 ± 0.02	
Digestive gland	**Atlantic**	12	1.41 ± 0.59	3.63 ± 2.03	54.02 ± 22.91	27.09 ± 12.02	2.37 ± 1.14	6.34 ± 3.61	0.36 ± 0.18	4.76 ± 1.98	
	Indian	18	1.56 ± 0.91	8.64 ± 4.13	54.90 ± 14.03	23.20 ± 6.57	4.99 ± 2.78	3.82 ± 1.17	0.96 ± 0.58	1.91 ± 0.54	
	Pacific	6	1.99 ± 0.52	12.50 ± 5.12	47.83 ± 11.22	27.99 ± 9.48	6.27 ± 1.79	2.03 ± 0.54	0.32 ± 0.06	1.02 ± 0.08	

DISCUSSION

Krill diet varied by region during the late-summer season as seen in their lipid, fatty acid and sterol biochemistry. Large differences in the shorter-term diet of krill can be seen in their stomach (days) and digestive gland (days-weeks) as assessed by neutral lipid fraction fatty acid profiles. Indian sector krill had a more diatomaceous and carnivorous based diet than krill in the Pacific and Atlantic sectors. Within each region, krill of different sexes showed little variation. The Indian sector was the exception, where sub-adult females and spent females had significantly different dietary signals. Differences in the diet of sex classes was predominantly due to a regional effect. Males and gravid females at Indian site 6 had vastly different diets, with females generally having higher DHA and males generally higher SDA.

Total lipid content

Atlantic sector digestive glands and whole krill samples showed much higher TL DM (mg g^{-1}) per krill than observed for the other regions. There were no significant differences between TL DM (mg g^{-1}) per krill between spent females and males. Earlier studies have reported large differences between krill sexes and TL content (Fricke et al., 1984, Mayzaud et al., 1998). However, sub-adult females were high in TL DM (mg g^{-1}) per krill, although lipid content decreased in gravid females and decreased further in spawned females in the Indian and Pacific sectors. This trend is not seen in females in the Atlantic sector. This difference may be due to primary production in the Atlantic sector being higher than in the Indian and Pacific sectors (El-Sayed and Weber, 1982, Vernet et al., 2008, Westwood et al., 2010).

The TL DM (mg g^{-1}) per krill in stomach samples was higher in the Pacific sector than in the Indian sector, despite Pacific sector krill digestive glands and whole krill sharing similar TL DM (mg g^{-1}) per krill as Indian sector samples. Indian sector krill may be utilising their digestive gland to store lipids (Dall et al., 1992, Virtue et al., 1993a). Krill stomachs digest and

break down lipids but they lack any short-term storage capability (Mayzaud et al., 1998, Schaafsma et al., 2017).

Changes in whole krill lipid variability has been shown to be related to variability in their digestive glands (Alonzo et al., 2005). Additionally, the fatty acid dietary signal observed in krill is due mostly to the dietary markers within the digestive gland (Alonzo et al., 2005). Stomach samples had lower TAG levels than the other tissue types, suggesting that this storage lipid is produced further down the digestive system (Mayzaud et al., 1998, Hagen et al., 2001). Krill stomachs are rarely used for lipid or fatty acid analysis due to their lack of TAG and neutral lipid fatty acids (Bottino, 1974, Ju and Harvey, 2004). These analyses are more commonly conducted on the digestive gland (for short term dietary signals) (Virtue et al., 1993a, Yoshida et al., 2009) which accumulates TAG during feeding. For longer-term dietary signals, whole krill are generally the preferred sample type used (Schaafsma et al., 2017, Ericson et al., 2018a, Hellessey et al., 2018). The use of neutral lipid fatty acid profiles of digestive and stomach tissue, rather than whole animal tissue, allows for even greater understanding of dietary components as it reflects the diet to a larger degree than fatty acids from phospholipids or total lipids.

Lipid class composition

Krill from the Indian sector had low PL levels in their digestive glands, but high PL levels in their whole body, showing a need for krill in this region to both metabolise and synthesise this lipid class. Pacific sector krill had high PL levels in their stomach and digestive glands and lower PL levels in their whole body, compared to krill from the other regions. Krill conserve PL and TAG based on their available dietary choices, the season, and their health status (e.g. PL is conserved until it is at required levels for adequate health, then TAG is stored) (Hagen et al., 1996, Hagen et al., 2001).

Stomachs and digestive glands had higher FFA as the organs where dietary items (such as lipids) are broken down (Henderson et al., 1981, Pond et al., 1995). Our results show higher FFA in the stomach than the digestive gland in two regions (Indian and Pacific sectors), which may be a function of the higher residence time of food in stomachs as a result of reduced feeding activity (Virtue et al., 1993a). The FFA were extremely high in Pacific sector krill stomach and digestive gland samples and this may be due to these samples being stored at -20 °C whereas krill from the Indian and Atlantic sectors were stored at -80 °C. This may mean that some enzymatic activity was still present in the Pacific sector krill samples as they were stored differently. However, whole krill samples from the Atlantic sector also had high FFA present, despite the FFA being low in their stomach and digestive gland samples. This potentially shows two different outcomes: 1) that the enzymatic activity even at -80 °C is still present in some tissues, or 2) that high FFA were present in samples regardless of the difference in storage temperatures.

Lipid class composition varied between the regions for krill digestive gland and stomach samples, but whole krill samples had a consistent lipid class composition within all regions. Whole krill lipids would be consistent for lipid classes required for reproduction, and growth, which would be similar in all regions (Pond et al., 1995, Ju and Harvey, 2004, Kohlbach et al., 2015), whilst stomach and digestive gland lipids would fluctuate greatly based on dietary input.

Lipid class composition by sex

Sub-adult females were generally higher in TAG percentage levels than gravid and spent females within the same region. Sub-adult females may be storing more fats going into their second winter (Atkinson et al., 2002). Older females that are spawning have used and are still using these stored lipids to reproduce (Mayzaud et al., 1998, Atkinson et al., 2002). Hence, older females have less TAG available and this was particularly evident in Indian sector krill samples. A large decrease in TAG could be due to 2016 being a less productive year (Bestley

et al., 2018, Schallenberg et al., 2018), so stored fats were being more readily used for reproduction in both mature females and males (Virtue et al., 1993a). Sub-adult females may also be outcompeting mature females and males for the food that is available (Atkinson et al., 2002).

Gravid female krill from the Indian and Atlantic sectors had higher sterol and lower PL percentage levels than spent females in the same region. Higher sterol levels in gravid females would help facilitate females moving lipids from their internal lipid stores into their eggs before spawning (Tarling et al., 2009b). Krill eggs are high in lipids, particularly sterols (Pond et al., 1995, Mayzaud et al., 1998), which accounts for the high sterol levels in gravid females. Atlantic sector male krill had much higher sterol levels than male krill in other regions due to energetic differences (Virtue et al., 1996).

Male and sub-adult female krill had significantly higher percentages of TAG than spent and gravid females and this may be why their PUFA levels are lower. Gravid female krill in particular do not store non-reproductive lipid (Mantel, 1983) and spent females are low in lipid (Mayzaud et al., 1998). Gravid females had large differences in their PUFA levels depending on the diet and productivity level in different regions, whilst sub-adult females and males show large differences in their PUFA levels in areas of lower production (Pacific and Indian sectors). MUFA levels were high in gravid females as well as sub-adult females and males in the Indian sector compared to other regions, further showing there is a major difference in their diet in this region.

These differences in diet can be seen more clearly once fatty acid markers and sterols are used to tease apart specific prey item groups within the digestive glands and stomachs. It is becoming more common for neutral lipid fractions to be utilised in analysis to see clearer dietary signals (Cabrol et al., 2019). Using only the fatty acids from the neutral lipid fractions

ensures that any signal from the structural cells of the digestive gland and stomach are not included into the dietary marker profile (Virtue et al., 1993b, Yoshida, 2009, Cabrol et al., 2019).

Diatoms and dinoflagellates

The fatty acid and sterol profiles revealed fine scale differences between regions. Digestive gland fatty acid profiles did not show as clear of a distinction between regions as the stomach sample profiles. This may be due to digestive glands being used for lipid storage within the body, so some markers could be stored here long after the shorter-term diet of the krill in that region has changed (Virtue et al., 1993a, Mayzaud et al., 1998, Yoshida et al., 2009). Fatty acid profiles from Atlantic and Pacific sector whole krill suggest they have a similar long-term diet despite their stomach profile being more unique. The storage of particular fatty acids in the digestive gland could be driving this similarity between the two regions. Stomachs showed the clearest distinction between regions, with krill from the Indian sector having a more carnivorous and diatomaceous diet and krill from the Atlantic and Pacific being higher in herbivory and flagellate markers.

Sterol levels (expressed as % of total sterols) in the stomach showed that diatoms (proposed source of 24-methylenecholesterol (Phleger et al., 2002)) were nearly double the dietary input in the Atlantic sector compared to the Pacific sector and 1.5 times that of the Indian sector, consistent with the fatty acid markers. Digestive gland sterol levels showed that this trend was amplified, as Atlantic sector krill showed nearly threefold higher levels of the diatomaceous sterols compared to Pacific sector krill and double that of the Indian sector krill. Fricke et al. (1984) showed that krill sterols were dominated by cholesterol and that most of the other major sterol markers (such as desmosterol) came from diatoms and other phytoplankton inputs. Phleger et al. (2002) reported that these algal sterol levels fluctuated

interannually and seasonally just like algal fatty acid markers as shown in Fricke et al. (1984) and Ericson et al. (2018a).

The essential and health-benefitting omega-3 (EPA (a diatom marker), DHA (a dinoflagellate marker) and SDA (a flagellate marker)) (Virtue et al., 1996, Ross and Quetin, 2000, O'Brien et al., 2011) were present at similar relative levels in all regions as they are used for krill growth and reproduction. Interestingly, Pacific sector krill had very high EPA, but was consistently lower in other diatom markers (16:1n-7c, 14:0 and 16:4n-1). These krill may be preferentially storing EPA, and using other, more readily metabolized fatty acids, for reproduction (Gigliotti et al., 2011), which is also seen in North Atlantic krill (Saether et al., 1986).

Omega-3 fatty acid levels in whole krill normally reflect the environmental omega-3 levels in that region (Hagen et al., 2001, Schaafsma et al., 2017). Higher environmental omega-3 levels might be driving krill in the Atlantic to conserve higher body levels, as this region has both diatoms and dinoflagellates in abundance in the late summer period (Korb et al., 2005). Atlantic sector krill also had higher PL levels in their stomachs, which may be due to an abundance of dietary items which are high in PL in this region (e.g. diatoms and dinoflagellates).

Indian sector krill had lower levels of omega-3 fatty acids in all tissue types at sites 4 and 5 and only DHA was elevated at site 6. The elevated levels of DHA at this site may be due to these krill being mostly spent females, whereas sub-adult females were analysed from sites 4 and 5. Males and gravid females from Indian site 6 had elevated DHA and SDA levels, consistent with a more dinoflagellate based diet at this more inshore site (El-Sayed and Weber, 1982, Korb et al., 2005, Ducklow et al., 2007). Male krill from Indian site 6 had higher DHA, SDA and 16:4n-1 showing a more herbivorous diet than males from the other Indian sector

sites. Pacific sector males were low in 16:0 (palmitic acid) and phytol (chlorophyll side-chain) levels, suggesting a more complex phytoplankton diet than occurs for the Indian and Atlantic sector male diets.

Atlantic and Pacific sector sub-adult females had mostly flagellate fatty acid markers (very high SDA), whilst Indian sector sub-adult females had a predominantly diatomaceous diet. Spent females were especially high in diatom and flagellate marker levels in the Indian sector (EPA and DHA). Their copepod to diatom ratio suggests an almost entirely phytoplankton-based diet. This may be due to the vital omega-3 fatty acids used for egg production (Mayzaud et al., 1998, Ross and Quetin, 2000, Tarling et al., 2009b) being found in diatoms and dinoflagellates and spent females needing to replenish these losses as quickly as possible once the eggs are spent. However, spent females in the Pacific sector had a diet that was lower in phytoplankton and spent females in the Atlantic sector showed a mixed diet with some phytoplankton markers (DHA, SDA, 16:4n-1 and palmitic acid). These varied strategies show that spent females are opportunistic and may be feeding on anything available to them as they must quickly build up fat reserves throughout the autumn to prepare for winter when krill use up most of their storage fats (Hellessey et al., 2018). The differences between gravid females could be due to the Pacific sector having lower levels of primary production and therefore to compensate, krill are needing to eat more detritus and marine snow (Schmidt et al., 2011).

Marine snow and detritus

Atlantic sector krill exhibited higher MSI levels in their digestive glands than their stomachs, potentially showing temporal effects of feeding on detrital type material. Marine snow marker percentages were higher in Pacific sector krill stomachs than Indian sector krill stomachs, reflecting marine snow levels in the Indian sector during summer (Turner, 2002, Stübing et al., 2003, Pasquer et al., 2010, Turner, 2015). Atlantic sector males were lower in marine snow

markers and spent females in the Pacific sector had a diet that was higher in marine snow and copepod marker levels.

Copepod and carnivory markers

Copepod marker levels were high in all Atlantic sector krill tissue types, indicative of a consistent level of feeding and metabolism of these fatty acid markers; e.g. no storage in the digestive gland is occurring for these markers. Copepods may be a more consistent food source for krill in the Atlantic sector (Ward et al., 2012b) and hence krill in this sector can maintain higher levels of these markers. Krill in the Indian and Pacific sectors, however, appear to be eating more copepods at this time of year; as indicated by higher copepod marker masses and levels in their stomachs and digestive glands than Atlantic sector krill (Chiba et al., 2001, Belcher et al., 2017, Schaafsma et al., 2017). In this study Indian sector spent females were low in copepod marker levels and their copepod to diatom ratio suggests an almost entirely phytoplankton-based diet. Carnivory markers, however, were found throughout all body tissues in all regions, with slightly higher levels in krill sampled from the Pacific sector. This may be due to cod-end feeding from the method of collection in the Pacific sector, a Bongo net (Morris et al., 1984), unlike the Atlantic sector collection method (a continuous pump). Carnivory has always been seen in low levels within krill diets regardless of where, when and how the studies were conducted (Ju and Harvey, 2004, Schmidt and Atkinson, 2016, Ericson et al., 2018a). Indian sector spent females were low in carnivory marker levels.

This study has revealed, that lipid class, neutral lipid fraction fatty acids and sterol dynamics in krill are variable depending on the region, sex and tissue type of the krill examined. Differences found in the total lipid, lipid class and neutral lipid fraction fatty acid and sterol composition and content, could be due to the varied trophic environments within each region. Future work could focus on 6 key fatty acids for dietary analysis between regions: EPA, DHA, SDA, 16:1n-7c, 16:4n-1 and 14:0. Examination of these fatty acids would allow greater

comparisons between diatomaceous and flagellate based diets in each region. Whilst MSI and copepod markers did influence krill diet, these had a smaller overall impact than the 6 major fatty acids listed above. Similarly, there was no clear trend between krill sex classes, however, sub-adult females were more carnivorous than mature krill. Future work looking into sexual maturity and krill diet could focus on the 18:1n-9c to 18:1n-7c ratio and copepod markers. Clarifying regional trends in krill diet will require further, more extensive, studies in all ocean basins – preferably throughout the year. Samples from the krill fishery offer the best opportunity for examining detailed seasonal and regional differences (Ericson et al., 2018a, Hellessey et al., 2018) although the fishery is currently regionally constrained. The standardised collection of krill from all ocean basins may be possible in the future due to an increase of research effort in the Ross Sea and the expanding fishery in the Indian sector of the Southern Ocean, allowing for greater dietary comparisons between these regions.

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Chapter 4: Antarctic Krill (*Euphausia superba* Dana 1850) Lipid and Fatty acid Content Variability is associated to Satellite Derived Chlorophyll a and Sea Surface Temperature in the Scotia Sea

This Chapter is currently under review at Nature Scientific Reports.

ABSTRACT

Antarctic krill (*Euphausia superba*) is a key component of the Antarctic food web with considerable lipid reserves that are vital for both their own and higher predator survival. Krill lipids are primarily derived from their diet of plankton, in particular diatoms and flagellates, but few attempts have been made to link the spatial and temporal variations in krill lipids to those in their food supply. Remotely-sensed environmental parameters provide large-scale information on the potential availability of krill food, although relating this to physiological and biochemical differences has only been performed on small scales and with limited samples. Our study utilised remotely-sensed data (Chlorophyll *a* and sea surface temperature) coupled with krill lipid data obtained from 3 continuous years of fishery-derived samples. We examined within and between year variation of trends in both the environment and krill biochemistry.

Chlorophyll *a* levels were positively related to krill lipid levels, particularly triacylglycerol which is a storage lipid. Plankton fatty acid biomarkers analysed in krill (such as n-3 polyunsaturated fatty acids) increased with decreasing sea surface temperature and increasing chlorophyll *a* levels. Our study demonstrates the utility of combining remote-sensing and fisheries data in examining biological and physiological relationships between Antarctic krill and the Southern Ocean environment.

INTRODUCTION

Antarctic krill (*Euphausia superba*, hereon krill) are at the centre of the wasp-waisted Southern Ocean ecosystem (Hill et al., 2006, Murphy et al., 2007). Krill, due to their high lipid (oil) content (up to 40% dry mass (Clarke, 1984, Atkinson et al., 2002)), are vital food for predators in the region (Stübing et al., 2003, Saunders et al., 2015). Krill have a naturally varied diet ranging from copepods and phytoplankton such as diatoms and flagellates, to marine snow and even cannibalism in harsh winter conditions (Hagen et al., 1996, Atkinson et al., 2002, Ju and Harvey, 2004, Ericson et al., 2018a). Krill are predominantly herbivorous during the summer and are more omnivorous from autumn to spring (Ericson et al, 2018a). Krill diet has been assessed through several different means such as microscopy (Schmidt and Atkinson, 2016), DNA extraction (Passmore et al., 2006, Töbe et al., 2010) and the use of signature fatty acid biomarkers (Ju and Harvey, 2004, Ericson et al., 2018a).

Biomarkers, such as fatty acids, have been used to examine krill health and diet previously (Bottino, 1974, Fricke et al., 1984, Virtue et al., 1993a, Mayzaud, 1997, Ju and Harvey, 2004, Kohlbach et al., 2015, Schaafsma et al., 2017, Ericson et al., 2018a), as they are a reliable way of looking at the long-term diet of krill (Passmore et al., 2006, Töbe et al., 2010, Schmidt and Atkinson, 2016). Fatty acid biomarkers are useful as they not only broadly classify what krill are eating, but their relative and absolute amounts allow insights into how much of these prey items and types krill have consumed over a more extended period of time (Ericson et al., 2018a, Ericson et al., 2018b, Hellessey et al., *in review*). Omega-3 (n-3) long-chain ($\geq C_{20}$) polyunsaturated fatty acids (n-3 LC-PUFA) are mainly derived from phytoplankton (Nichols et al., 1986, Butler, 2007), and are needed for krill health, growth and reproduction. They also serve as useful biomarkers (Virtue et al., 2010, O'Brien et al., 2011, Ericson et al., 2018a) in food-chain research. In particular, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), which are known to be associated with the intake of diatoms and

dinoflagellates respectively (Bottino, 1974, Ericson et al., 2018a), are needed for production of krill eggs before spawning, and for the development of the larval krill (Yoshida, 2009). EPA and DHA are consistently abundant in krill and make up a large part of the krill fatty acid profile (Kolakowska et al., 1994, Ericson et al., 2018a). Other sources for EPA and DHA may also exist. The specific source(s) of other n-3 LC-PUFA such as eicosatetraenoic acid (ETA, 20:4n-3) and docosapentaenoic acid (DPA, 22:5n-3) are not as well defined. EPA and DHA are consistently abundant in krill and make up a large part of the krill fatty acid profile (Kolakowska et al., 1994, Ericson et al., 2018a), particularly in summer and autumn. EPA and DHA are also the n-3 LC-PUFA targeted by the krill fishing industry for application into nutraceutical products (Nicol et al., 2012, Hill, 2013, Schutt, 2016). The n-3 PUFA, stearidonic acid (SDA, 18:4n-3), also plays a vital role in krill diet, although its precise function is not well understood (Ericson et al., 2018a).

Phytoplankton, such as diatoms and dinoflagellates, all naturally produce chlorophyll which can be remotely detected via satellites (Moore and Abbott, 2002, Johnson et al., 2013, Zeng et al., 2016) using ocean colour data. Recent studies have shown that the colour of the ocean, due to shifts in the assemblage of these phytoplankton blooms (Deppeler and Davidson, 2017), is changing with climate change (Dutkiewicz et al., 2019). As these phytoplankton assemblages change, krill diet may also be altered as the climate changes (Deppeler and Davidson, 2017, Hancock et al., 2018). Phytoplankton biomarker levels will have more pronounced changes within krill diet as lower trophic level populations will shift more rapidly with climate change (Deppeler and Davidson, 2017) than higher trophic levels (Fraser and Hofmann, 2003), such as grazers like copepods and krill (Schofield et al., 2010). Therefore, the biochemical composition of krill will shift year round from that of a seasonal winter diet which includes copepods (omnivorous) to a summer diet (herbivorous) (Ericson et al., 2018a) for a larger part of the year as sea ice is lost and waters warm (increase in sea surface temperature, SST) and

become more acidic, allowing for greater phytoplankton blooms to occur (Montes-Hugo et al., 2009, Behrenfeld et al., 2017, Deppeler and Davidson 2017). It is difficult, however, to link changes in the marine environment to changes in krill biochemistry *in situ*. Controlled aquarium experiments are difficult to conduct over longer time scales and cannot emulate conditions over large geographic areas. Understanding these large-scale relationships requires data collected over wide areas and long timeframes. Hence, using remotely-sensed satellite chlorophyll *a* (Chl *a*) data as a proxy for primary production is optimal for data collection that can happen simultaneously over large geographic areas and over span long timeframes (Zeng et al., 2016, Behrenfeld et al., 2017). Similarly, SST is a major environmental driver of the phytoplankton blooms during spring and summer in the Southern Ocean (El-Sayed and Weber, 1982, Helbling et al., 1995, Moline et al., 1997, Garibotti et al., 2005); increases in SST are a major cause of ecosystem level shifts (e.g. phytoplankton assemblage change) with climate change (Montes-Hugo et al., 2009, Behrenfeld et al., 2017, Deppeler and Davidson, 2017). Remotely-sensed SST data from satellites can also be collected over large geographic areas and long timeframes. Unfortunately, as of yet, a proxy for zooplankton, marine snow or bacterial assemblages that can be detected via satellite are not available. However, biological and biochemical data from krill samples are able to be collected and analysed over a large region of the Southern Ocean and over a long timeframe by the krill fishery, such as in Tarling, et al. (2016a).

The krill fishery has been operating since the early 1970's (Nicol and Foster, 2016), and in the mid-1990s the industry began to produce and sell krill oil as a nutraceutical due to its high levels of omega-3 containing oils (Nicol et al., 2012). The krill fishery operates year-round in the South Atlantic Ocean (Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) Area 48) and in particular near the West Antarctic Peninsula (WAP, CCAMLR Sub-Area 48.1), South Orkney Islands (SOI, CCAMLR Sub-Area 48.2) and

South Georgia (SG, CCAMLR Sub-Area 48.3). Over the last few years the total catch has been approximately 300,000 tonnes of krill a year, and from a scientific research perspective the commercial harvest is a useful source of biological samples (Tarling et al., 2016a, Ericson et al., 2018a, Hellessey et al., 2018). Satellites collect environmental data such as SST, ocean colour, sea surface height, fluorescence, wind direction and wind speed. While satellite data must be calibrated and validated, it can fill in gaps of data collected *in situ* and provides wide-area coverage. Linking remotely-sensed environmental data with analysis of samples collected by the krill fishery is a cost-effective approach to explore biological responses to environmental changes.

Our study uses three consecutive years of krill lipid data from fishery-derived samples collected throughout the South Atlantic Ocean. We simultaneously accessed satellite data to link krill signature biochemical data to environmental conditions seen at the location of krill collection. We hypothesise that changes in krill biochemistry, specifically their fatty acid dietary biomarkers, will track broad scale satellite-derived environmental data. Linking environmental drivers to krill diet will assist in ecosystem energy budget and food web models. By basing such models around environmental parameters, future environmental scenarios can be modelled, and krill diet and ecosystem responses will be more reliably predicted.

We aim to show if a link between the environment and krill diet exists by examining whether: 1) chlorophyll *a* levels (Chl *a*, mg m^{-2}), total lipid (mg g^{-1} krill dry mass) and phytoplankton fatty acid biomarkers (percentage (%) and mass (ug)) increase as SST (°C) decreases throughout summer and autumn, post the annual spring phytoplankton blooms; 2) larger shifts in SST and Chl *a* closer to the pole (currently an area in a state of flux) will drive larger shifts in krill biochemistry and 3) SST and Chl *a* is correlated to total lipid or phytoplankton fatty acid biomarkers in a meaningful manner.

METHODS

Krill sample collection and analysis

Krill lipid and fatty acid data used for this analysis are published in Hellessey et al. (2018) and Ericson et al. (2018a). Briefly, samples were collected by the FV *Saga seas* using a continuous underwater pumping system in the South Atlantic Ocean (Area 48). Samples were collected from January 2014 – September 2016 in the WAP (Sub-Area 48.1), the SOI (Sub-Area 48.2), and SG (Sub-Area 48.3). Krill were stored at -80°C and transported on dry ice to Hobart, Tasmania for lipid and fatty acid analysis.

Three adult male and female krill were analysed each fortnight, and these lipid and fatty acid profiles were pooled (N=6 per fortnight, total N = 391). Samples were quantitatively extracted overnight using a modified Bligh and Dyer (1959) method as described in Hellessey et al. (2018) to produce the total solvent extract (TSE). The total lipid content (TL, expressed as mg) of each sample was weighed gravimetrically in a pre-weighed 2 ml glass vial. To account for differences in krill size, the TL was divided by krill dry mass (g) and is expressed as mg of total lipid content per gram of krill dry mass (mg g^{-1}, TL DM). Lipid class composition was determined by analysis of the TSE on an Iatroscan TLC-FID analyser following Hellessey et al. (2018). Aliquots of sample TSEs were methylated to produce the fatty acid methyl esters (FAME) of the sample (Ericson et al. 2018a). Samples were made up with 1 ml internal injection standard (23:0 FAME) and analysed by gas chromatography (GC-FID) (Iverson et al., 2004). Samples were injected (0.2 µl) and the identification and quantification of fatty acids (expressed as % total fatty acid area) was conducted in comparison to a commercial standard mix (sourced from Sigma) and a known laboratory standard (tuna oil). Fatty acid identifications were further confirmed through gas chromatography-mass spectrometry (GC-MS) analyses (Iverson et al., 2004).

Satellite data extraction and analysis

This study used ocean colour data from the NASA Moderate Resolution Imaging Spectroradiometer Aqua (MODIS-Aqua) L3 mapped data products (https://oceancolor.gsfc.nasa.gov/data/aqua/) and sea surface temperature data from the GHRSST L4 gridded products (https://data.nodc.noaa.gov/ghrsst/L4/). The sea surface temperature data and the 3 different ocean colour Remote Sensed Reflectance wavelengths (RRS; red, green and blue: 443, 488 and 555 nm, respectively) were extracted for each date krill were collected in an area in the south Atlantic Ocean (bounds of 55-80 °S and 30-80 °W) in a 1 km x 1 km grid of pixels. The exact GPS location of krill collection on that date was then used to extract the pixel value (28 successful matches = 4.18% matched, Table 4.1), but due to the low match-up rate this was expanded both temporally and spatially to an 8-day (8D) average and a 3 km x 3 km (3x3) pixel area (145 matches = 21.64% matched, Table 4.1). Whenever data was patchy, it was smoothed linearly to the nearest pixel within a 4 km area.

Table 4.1: Decision table for why we increased our temporal and spatial fields for the red, green and blue wavelengths in Moderate Resolution Imaging Spectroradiometer (MODIS) to generate chlorophyll a data. The Commission for Conservation of Antarctic Marine Living Resources (CCAMLR) regions were defined as the West Antarctic Peninsula (WAP, Area 48.1), the South Orkney Islands (SOI, Area 48.2) and South Georgia (SG, Area 48.3) (www.ccamlr.org). Raw value for percent data match in brackets. Total number of days with lipid data to match against = 670.

Case	Temporal averaging	Pixel averaging	Percentage (%) match
1	Daily	1 km x 1 km	4.18 (28)
2	Daily	3 km x 3 km	7.01 (47)
3	8 Day	1 km x 1 km	21.64 (145)
4	8 Day	3 km x 3 km	27.91 (187)
5	8 Day	Custom CCAMLR Regions	WAP - 46.26 (310) SOI - 51.34(344) SG - 66.86 (448)

The ocean colour RRS data were converted into Chl *a* concentrations using the MODIS Southern Ocean chlorophyll algorithm in Johnson et al. (2013). Once converted into Chl *a* concentrations, this environmental data (SST and Chl *a*) was merged into the same data frame as the lipid data by matching the date and GPS location of 1 day 1x1 pixel locations to the date and GPS location of krill harvest. To examine the seasonal trend for Chl *a*, each CCAMLR fishing sub-area within the South Atlantic (Area 48) also had its Chl *a* calculated for 8-day averages. These wider geographic areas of the WAP, the SOI and SG generated much higher recovery rates for Chl *a* (46.26%, 51.34% and 66.86% respectively, Table 4.1) and is hereon called Chl *a* (CCAMLR). This data was also merged into the same data frame by matching dates and GPS locations of krill sampling, as done previously.

However, data for some dates did not match krill harvesting location (e.g. Chl *a* data from SG on a day when krill were collected from WAP), so the rates of Chl *a* recovery decreased once matched to krill lipid data. Therefore, to achieve the best Chl *a* matches to lipid data, the Chl *a* concentration was kept in a hierarchy from Case 1 to 5 (Table 4.1). For example, if a daily pixel match was available this was kept in preference over an 8-day 3x3 or 8-day regional Chl *a* concentration. This method increased the overall match rate to 226 matches = 60.59% matched. This merged Chl *a* data (hereon called Chl *a* (overall)) was used to examine the larger scale trends in Chl *a* across the entire south Atlantic Ocean and krill lipids.

Data and statistical analysis

Statistical analysis was done in RStudio (version 1.0.153 © 2017) using packages: nlme (Pinheiro et al., 2017), ggplot2 (Wickham, 2009), ggmap (Kahle and Wickham, 2013), maps (Becker, 2017), anytime (Eddelbuettel, 2018), and reshape2 (Wickham, 2007). Multifactorial ANOVAs were performed using SST, Chl *a*, as well as their interaction terms, as factors for the variables of total lipid content (mg g^{-1} dry mass), PL and TAG percentage, individual fatty acid percentage and mass data for the fatty acids most associated with primary production

(mostly diatom and flagellate markers), and the ratios of 16:1/16:0 to look at diatom levels (Ericson et al., 2018a) and EPA:DHA to look at diatom or flagellate dominance in the diet (Ericson et al., 2018a). Data were log or square root transformed when the assumptions of normality and homogeneity of variances were not met - Chl *a* is typically log distributed. Linear models were similarly produced using the same factors as for the multifactorial ANOVAs, including interaction terms, but sub-divided into summer/autumn and winter/spring models due to the large seasonal shift in SST arising from the change of harvesting location by the FV *Saga Seas*. Models were tested for fit using a standard regression table, where the adjusted r^2 value showed the fit of points to the confidence interval of the model. Models were additionally run through drop testing and Tuckey post-hoc tests to ensure no compounding of results was occurring. Models of best fit had adjusted r^2 values > 0.5, a P value of < 0.05 from the multifactorial ANOVAs and a χ^2 value above 0.1. These models are shown within all tables throughout the Results as greyed out.

Maps were produced within R using the maps and ggmap packages to see the geographic distribution of krill lipid content (mg g^{-1} dry mass) as well as SST and Chl *a*.

RESULTS

Seas Surface Temperatures

During this study (Jan 2014 – Sep 2016) satellite-derived sea surface temperatures had the greatest variability in the South Orkney Islands (SOI), ranging from -1.28 to 1.93 °C (average 0.33 ± 0.86 °C), followed by South Georgia (SG) where temperatures ranged from -1.10 to 1.49 °C (average 0.49 ± 0.54 °C) and the West Antarctic Peninsula (WAP) which ranged from -1.28 to 0.94 °C (average -0.25 ± 0.78 °C). Temperatures decreased from summer into autumn in the WAP and SOI, and from the start of winter to spring in SG. However, in 2016, SST at WAP increased from summer into autumn and then decreased from early autumn onwards (Figure 4.1).

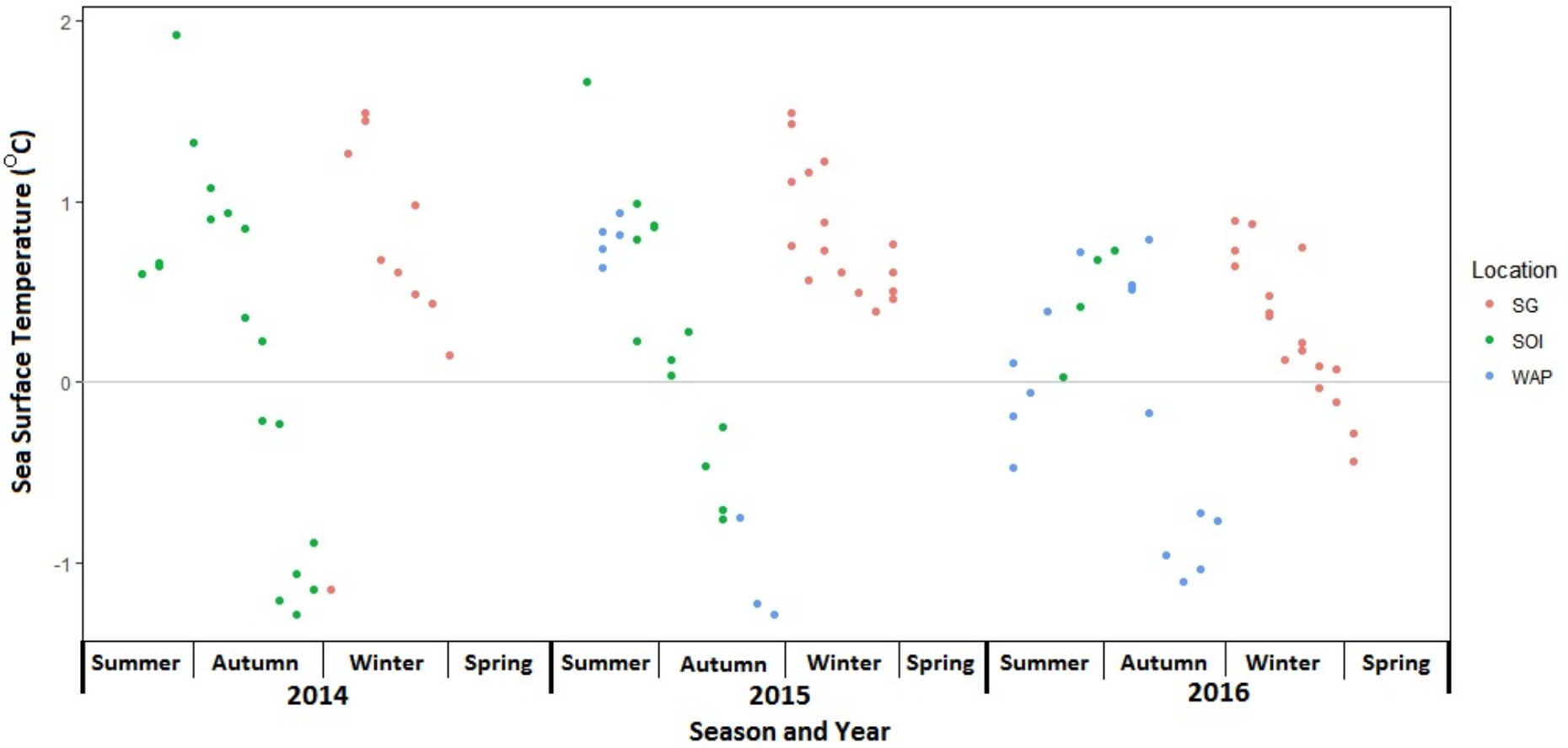

Figure 4.1: Sea surface temperatures (°C) from January 2014 – September 2016 coloured by *Euphausia superba* sample location (SG: South Georgia, SOI: South Orkney Islands, WAP: West Antarctic Peninsula). The x-axis is the season and year of krill sample collection.

Chlorophyll a concentrations

The levels of Chl *a* varied depending on the location and the season (Figure 4.2). Values of more than 5.78 mg m^{-2} of Chl *a* were observed in the SOI, and as low as 0.19 mg m^{-2} Chl *a* in SG. Chl *a* increased in summer and decreased rapidly during the autumn of 2014 around the SOI. Chl *a* concentrations in other years were more consistent, although very few satellite measurements were recorded at SG due to the time of year, cloud cover, ice cover and suboptimal sun angle.

Chl *a* varied the most around the SOI, ranging from 0.24 to 5.78 mg m^{-2} (average 0.88 ± 0.85 mg m^{-2}), followed by the WAP which ranged from 0.20 to 1.34 mg m^{-2} (average 0.62 ± 0.30 mg m^{-2}), and SG which ranged from 0.19 to 0.62 mg m^{-2} (average 0.39 ± 0.11 mg m^{-2}). SG had the smallest range of Chl *a* concentrations and the lowest average Chl *a* concentration. SOI had the biggest range of Chl *a* concentrations and the highest average Chl *a* concentration.

Geographic distribution

The geographic distribution of SST, Chl *a* and krill total lipid (mg g^{-1}) dry mass (TL DM), for CCAMLR Sub-Areas 48.1, 48.2 and 48.3 can be seen in Figures 4.3, 4.4 and 4.5. . Higher SST and Chl *a* in the Bransfield Strait (WAP) reflect bathymetric and current features (Fig. 4.3) as well as the northern advection of water from the deep canyon to the south of the SOI (Korb et al., 2005) (Fig. 4.4). These oceanographic features are known locations of higher SST and Chl *a* within the SG and SOI areas. Krill TL DM is also higher in the Bransfield Strait (Fig. 4.3). To the northwest of SG, higher SST and lower Chl *a* values can be seen due to the faster flowing current coming off of the slope of SG (Fig. 4.5).

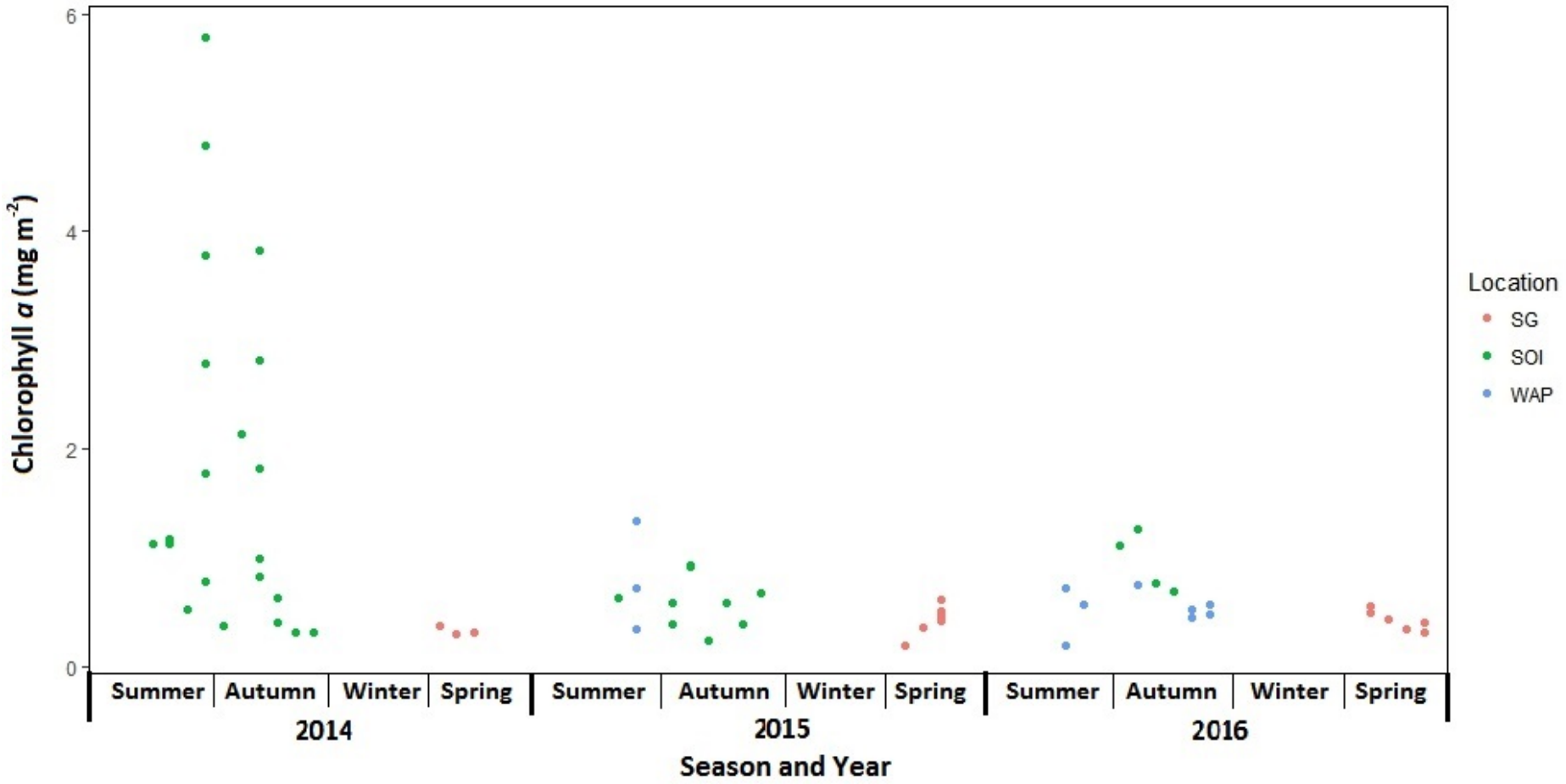

Figure 4.2: Chlorophyll *a* concentrations (mg m^{-2}) from January 2014 – September 2016 coloured by *Euphausia superba* sample location (SG: South Georgia, SOI: South Orkney Islands, WAP: West Antarctic Peninsula). The x-axis is season and year of krill sample collection.

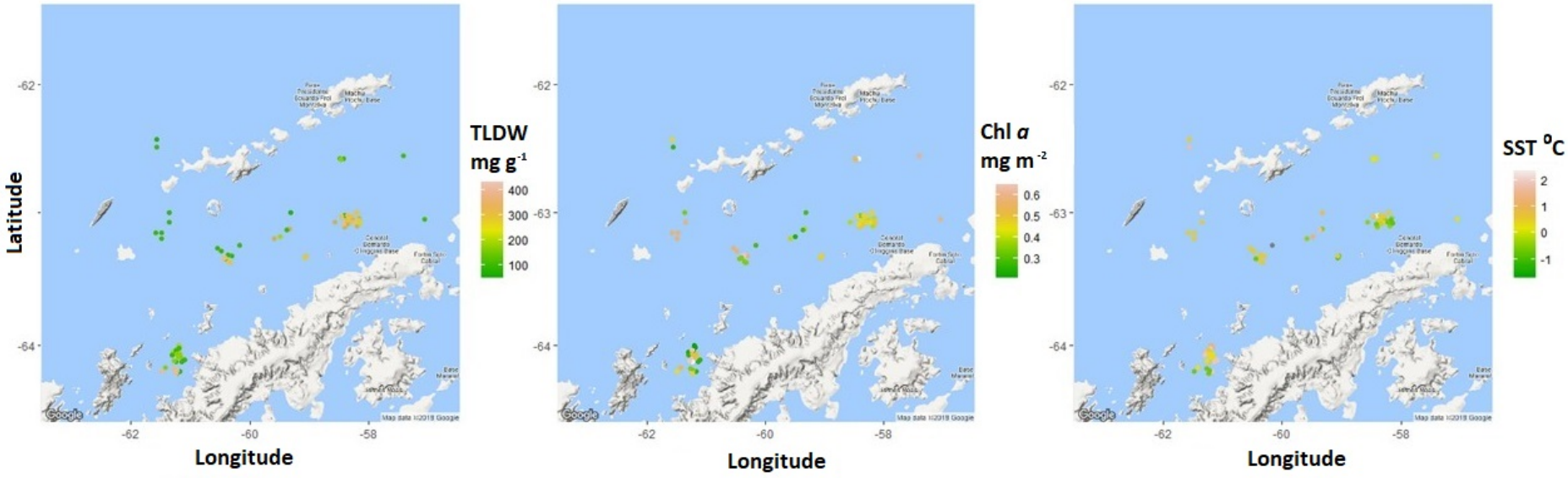

Figure 4.3: The geographic distribution of krill total lipid (mg g^{-1}) dry mass (TLDM), the chlorophyll *a* (Chl *a*) concentration (mg m^{-2}) and the sea surface temperature (°C, SST) of *Euphausia superba* samples collected in the West Antarctic Peninsula. Locations are points that krill were harvested by *FV Saga Seas* from January to May 2014 - 2016. Maps were produced using the RStudio (version 1.0.153 © 2017) package ggmaps (Kahle and Wickham, http://journal.r-project.org/archive/2013-1/kahle-wickham.pdf). Map data © 2018 Google.

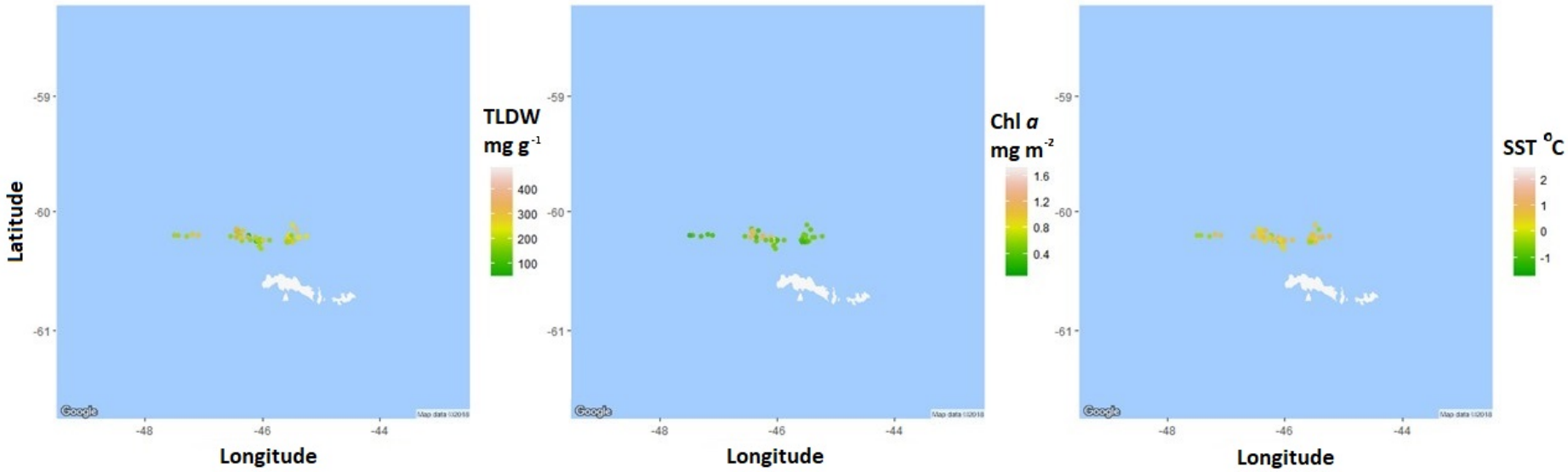

Figure 4.4: The geographic distribution of krill total lipid (mg g^{-1}) dry mass (TLDM), the chlorophyll *a* (Chl *a*) concentration (mg m^{-2}) and the sea surface temperature (°C, SST) of *Euphausia superba* samples collected in the South Orkney Islands. Locations are points that krill were harvested by *FV Saga Seas* from January to May 2014 - 2016. Maps were produced using the RStudio (version 1.0.153 © 2017) package ggmaps (Kahle and Wickham, http://journal.r-project.org/archive/2013-1/kahle-wickham.pdf). Map data © 2018 Google.

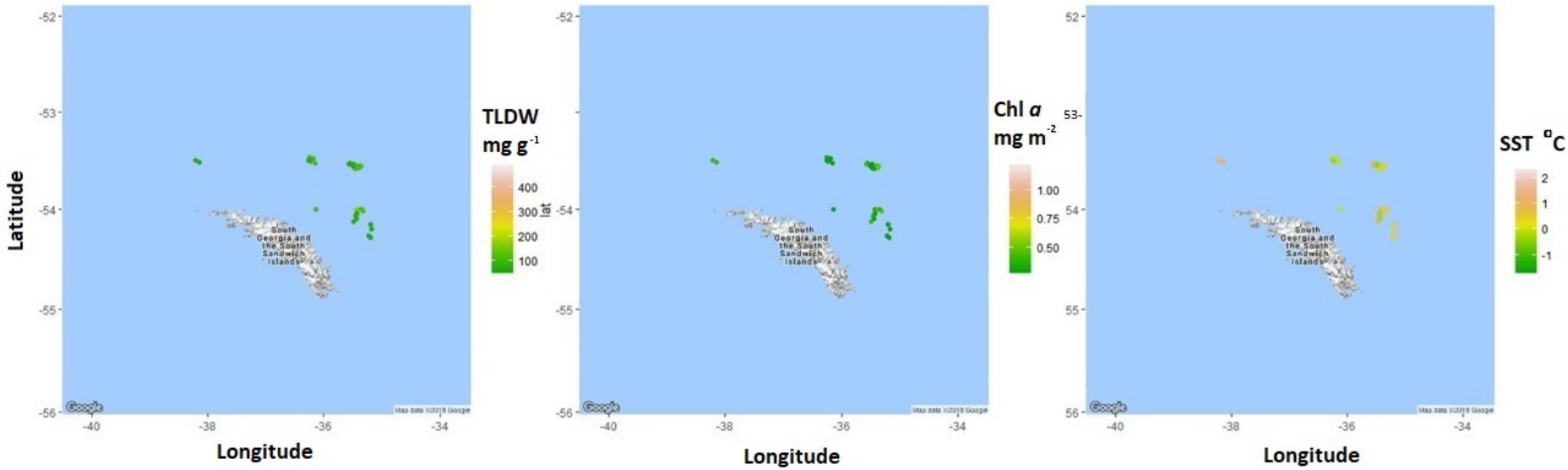

Figure 4.5: The geographic distribution of krill total lipid (mg g^{-1}) dry mass (TLDM), the chlorophyll *a* (Chl *a*) concentration (mg m^{-2}) and the sea surface temperature (°C, SST) of *Euphausia superba* samples collected at South Georgia. Locations are points that krill were harvested by *FV Saga Seas* from June to September 2014 - 2016. Maps were produced using the RStudio (version 1.0.153 © 2017) package ggmaps (Kahle and Wickham, http://journal.r-project.org/archive/2013-1/kahle-wickham.pdf). Map data © 2018 Google.

Lipid and fatty acid general trends

Results describing the trends seen in krill TL DM, lipid classes and their fatty acids can be found in Ericson, et al. [7] and Hellessey, et al. [46]. Briefly, krill TL DM and triacylglycerol (TAG) percentage (%) increased throughout summer to reach autumn highs in the WAP and SOI, whereas krill at SG had declining TL DM and TAG % throughout winter and spring. EPA and DHA (mg g^{-1} dry weight) followed the same seasonal trend as TL DM and TAG %. 16:1n-7c and SDA had variable quantities across all seasons, years and fishing locations. In summer, krill had high levels (% total fatty acids) of EPA, DHA and PUFA, but low 18:1n-9c/18:1n-7c ratios, indicating a more herbivorous diet.

Tracking fatty acid biomarkers using satellite derived environmental data

Clear seasonal trends can be seen in fatty acid biomarkers in krill throughout the fishing seasons both in percentage composition and quantitative amounts (mass, ug). Most diatom-based markers in krill (such as EPA, 16:4n-1 and 16:1n-7c) increased in percentage throughout summer to reach autumn highs in the WAP and SOI, as did 16:0 percentages, whereas krill at SG had declining diatom-based marker and 16:0 percentages throughout winter and spring. Dinoflagellate markers in krill such as DHA and SDA showed similar trends to diatom markers in their percentages. Both diatom and flagellate markers in krill showed the opposite trend in their masses (low in summer and autumn, higher in winter and spring) but this could be due to location of sampling (WAP/SOI in summer/autumn and SG in winter/spring). Table 4.2 provides the *p*-values for 1-way ANOVAs comparing models as well as the adjusted r^2 value and χ^2 value for the associated model of best fit between the krill's biochemical data (lipid and fatty acid content (mass) and composition (percentage)) and the environmental data (SST and Chl *a*) and their interaction terms from the South Atlantic region. Tables 4.3-4.5 show these same relationships broken down into the smaller CCAMLR management sub-areas (WAP, SOI and SG, respectively).

Table 4.2: *Euphausia superba* total lipid (mg g^{-1}) dry mass (TL DM), and lipid class composition (phospholipid (PL) and triacylglycerol (TAG) percentage) and fatty acid (20:5n-3 (EPA), 22:6n-3 (DHA), and 18:4n-3 (SDA)) percentage composition (%) and mass (ug) per krill against sea surface temperature (SST), chlorophyll *a* (Chl *a*) and their interaction terms for all seasons and pooled locations across the South Atlantic sector. Chl *a* was measured at both an overall scale (overall) and an 8-day 3 km x 3 km (8D 3x3) pixel scale for the entire South Atlantic sector. Values given are for: P values, r^2 values (italics) and χ^2 values (bold) for the model of best fit. Cells that are greyed out have a P value < 0.05, an r^2 of >0.5 and a χ^2 value > 0.1.

	SST	Chl *a* (overall)	Chl *a* (8D 3x3)	SST*Chl *a* (overall)	SST*Chl *a* (8D 3x3)
TL DM (mg g^{-1})	< 0.001 (*0.144*) **0.086**	0.948 (*-0.004*) **0.018**	0.434 (*-0.005*) **0.238**	0.808 (*0.045*) **0.082**	0.007 (*0.134*) **0.238**
PL %	0.296 (*0.000*) **0.003**	< 0.0001 (*0.061*) **0.006**	< 0.0001 (*0.255*) **0.250**	0.176 (*0.069*) **0.029**	0.074 (*0.376*) **0.249**
TAG %	0.001 (*0.026*) **0.001**	0.344 (*-0.000*) **<0.001**	0.035 (*0.043*) **0.087**	0.480 (*-0.006*) **0.007**	0.015 (*0.193*) **0.087**
EPA %	0.001 (*0.025*) **0.080**	0.426 (*-0.002*) **0.018**	0.006 (*0.078*) **0.241**	< 0.0001 (*0.085*) **0.241**	0.372 (*0.068*) **0.241**
EPA (ug)	< 0.001 (*0.167*) **0.239**	0.664 (*-0.004*) **0.239**	0.667 (*-0.010*) **0.238**	0.385 (*0.051*) **0.239**	0.058 (*0.074*) **0.238**
DHA %	< 0.001 (*0.065*) **0.082**	0.109 (*0.007*) **0.081**	0.194 (*0.009*) **0.240**	0.203 (*0.059*) **0.242**	0.918 (*-0.016*) **0.240**
DHA (ug)	< 0.001 (*0.158*) **0.239**	0.534 (*-0.003*) **0.239**	0.102 (*0.021*) **0.238**	0.851 (*0.067*) **0.239**	0.048 (*0.117*) **0.238**
SDA %	0.078 (*0.006*) **0.003**	0.626 (*-0.003*) **0.003**	0.446 (*-0.005*) **0.240**	0.013 (*0.024*) **0.082**	0.612 (*0.189*) **0.240**
SDA (ug)	< 0.001 (*0.141*) **0.239**	0.603 (*-0.003*) **0.239**	0.921 (*-0.012*) **0.238**	0.397 (*0.052*) **0.239**	0.422 (*0.096*) **0.238**
16:0 %	0.001 (*0.037*) **0.083**	0.588 (*-0.003*) **0.082**	0.943 (*-0.012*) **0.244**	0.034 (*0.034*) **0.243**	0.417 (*-0.018*) **0.244**
16:0 (ug)	< 0.001 (*0.182*) **0.239**	0.920 (*-0.004*) **0.239**	0.555 (*-0.008*) **0.238**	0.759 (*0.068*) **0.239**	0.091 (*0.042*) **0.239**
16:4n-1 %	0.925 (*-0.003*) **< 0.001**	0.451 (*-0.002*) **<0.001**	0.334 (*-0.000*) **0.019**	0.939 (*-0.010*) **0.003**	0.174 (*0.126*) **0.019**
16:4n-1 (ug)	< 0.001 (*0.103*) **0.239**	0.407 (*-0.001*) **0.239**	0.337 (*-0.000*) **0.238**	0.787 (*0.008*) **0.239**	0.899 (*0.125*) **0.238**
6:1n-7c %	0.115 (*0.004*) **0.082**	0.982 (*-0.004*) **0.080**	0.583 (*-0.009*) **0.241**	0.061 (*0.015*) **0.241**	0.989 (*-0.034*) **0.241**
16:1n-7c (ug)	< 0.001 (*0.143*) **0.239**	0.972 (*-0.004*) **0.239**	0.456 (*-0.005*) **0.238**	0.539 (*0.059*) **0.239**	0.069 (*0.043*) **0.238**
16:1/16:0 ratio (ug)	0.588 (*-0.002*) **0.239**	0.781 (*-0.004*) **0.239**	0.543 (*-0.008*) **0.238**	0.136 (*0.006*) **0.239**	0.950 (*-0.034*) **0.238**
EPA/DHA ratio (ug)	0.621 (*-0.002*) **0.239**	0.944 (*-0.004*) **0.239**	0.008 (*0.073*) **0.238**	0.730 (*-0.012*) **0.239**	0.625 (*0.060*) **0.238**
Phytanic acid %	0.013 (*0.014*) **<0.001**	0.047 (*0.013*) **<0.001**	0.149 (*0.014*) **<0.001**	0.309 (*0.012*) **<0.001**	0.362 (*0.098*) **<0.001**
Phytanic acid (ug)	0.009 (*0.016*) **<0.001**	0.052 (*0.012*) **<0.001**	0.149 (*0.014*) **<0.001**	0.313 (*0.009*) **<0.001**	0.250 (*0.127*) **<0.001**

Table 4.3: Euphausia superba (collected from the West Antarctic Peninsula) total lipid (mg g^{-1}) dry mass (TL DM), lipid class (phospholipid (PL) and triacylglycerol (TAG)) and fatty acid (20:5n-3 (EPA), 22:6n-3 (DHA) and 18:4n-3 (SDA)) percentage composition (%) and mass (ug) in relation to sea surface temperature (SST), chlorophyll *a* (Chl *a*) and their interaction terms. Chl *a* was measured at both a Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) region wide scale (CCAMLR region) and at an 8-day 3 km x 3 km (8D 3x3) pixel scale. Values given are for: P values, r^2 values (italics) and χ^2 values (bold) for the model of best fit. Cells that are greyed out have a P value < 0.05, an r^2 of >0.5 and a χ^2 value > 0.1.

	SST	Chl *a (CCAMLR)*	Chl *a (8D 3x3)*	SST*Chl *a (CCAMLR)*	SST*Chl *a (8D 3x3)*
TL DM (mg g^{-1})	< 0.001 (*0.304*) **0.240**	0.014 (*0.148*) **0.238**	0.185 (*0.059*) **0.235**	0.001 (*0.363*) **0.238**	0.002 (*0.610*) **0.235**
PL %	0.006 (*0.077*) **0.028**	0.004 (*0.212*) **0.089**	0.949 (*-0.071*) **0.235**	< 0.001 (*0.564*) **0.089**	0.001 (*0.655*) **0.235**
TAG %	0.007 (*0.073*) **0.102**	0.001 (*0.277*) **0.023**	0.782 (*-0.065*) **0.235**	< 0.001 (*0.559*) **0.089**	0.004 (*0.568*) **0.235**
EPA %	0.001 (*0.137*) **0.240**	0.012 (*0.157*) **0.238**	0.388 (*-0.014*) **0.235**	< 0.001 (*0.559*) **0.238**	< 0.001 (*0.689*) **0.235**
EPA (ug)	< 0.001 (*0.217*) **0.239**	0.079 (*0.065*) **0.238**	0.059 (*0.175*) **0.235**	0.001 (*0.311*) **0.238**	0.002 (*0.615*) **0.235**
DHA %	0.001 (*0.122*) **0.239**	0.254 (*0.010*) **0.238**	0.001 (*0.498*) **0.235**	0.028 (*0.148*) **0.238**	0.014 (*0.467*) **0.235**
DHA (ug)	< 0.001 (*0.285*) **0.239**	0.357 (*-0.004*) **0.238**	0.624 (*-0.053*) **0.235**	< 0.001 (*0.430*) **0.238**	< 0.001 (*0.892*) **0.235**
SDA %	0.795 (*-0.011*) **0.083**	0.187 (*0.024*) **0.086**	0.009 (*0.351*) **0.235**	0.541 (*0.098*) **0.087**	0.068 (*0.295*) **0.235**
SDA (ug)	< 0.001 (*0.311*) **0.239**	0.020 (*0.131*) **0.238**	0.372 (*-0.010*) **0.235**	0.001 (*0.390*) **0.238**	0.004 (*0.560*) **0.235**
16:0 %	0.001 (*0.122*) **0.240**	0.048 (*0.089*) **0.238**	0.012 (*0.325*) **0.235**	0.014 (*0.214*) **0.238**	0.014 (*0.464*) **0.235**
16:0 (ug)	< 0.001 (*0.254*) **0.239**	0.059 (*0.078*) **0.238**	0.097 (*0.126*) **0.235**	0.001 (*0.347*) **0.238**	0.002 (*0.621*) **0.235**
16:4n-1 %	0.527 (*-0.007*) **0.021**	0.945 (*-0.031*) **0.245**	0.004 (*0.412*) **0.235**	0.982 (*0.189*) **0.245**	0.003 (*0.589*) **0.235**
16:4n-1 (ug)	< 0.001 (*0.197*) **0.239**	0.059 (*0.079*) **0.238**	0.041 (*0.213*) **0.235**	0.008 (*0.280*) **0.238**	0.093 (0.253) **0.235**
16:1n-7c %	0.130 (*0.015*) **0.240**	0.625 (*-0.023*) **0.240**	0.003 (*0.446*) **0.235**	0.104 (*0.043*) **0.241**	0.026 (*0.406*) **0.235**
16:1n-7c (ug)	< 0.001 (*0.185*) **0.239**	0.083 (*0.063*) **0.238**	0.080 (*0.145*) **0.235**	0.001 (*0.302*) **0.238**	0.014 (*0.468*) **0.235**
16:1/16:0 ratio (ug)	0.445 (*-0.005*) **0.239**	0.897 (*-0.031*) **0.238**	0.001 (*0.489*) **0.235**	0.192 (*0.012*) **0.238**	0.017 (*0.449*) **0.235**
EPA/DHA ratio (ug)	0.031 (*0.043*) **0.239**	0.181 (*0.026*) **0.238**	0.003 (*0.436*) **0.235**	0.188 (*0.162*) **0.238**	0.040 (*0.358*) **0.235**
Phytol %	0.426 (*-0.004*) **<0.001**	0.013 (*0.156*) **<0.001**	0.859 (*-0.074*) **<0.001**	0.039 (*0.246*) **<0.001**	0.072 (*0.308*) **<0.001**
Phytol (ug)	0.816 (*-0.011*) **<0.001**	0.014 (*0.149*) **<0.001**	0.799 (*-0.066*) **<0.001**	0.145 (*0.185*) **<0.001**	0.046 (*0.342*) **<0.001**

Table 4.4: Euphausia superba (collected from South Orkney Islands) total lipid (mg g^{-1}) dry mass (TL DM), lipid class (phospholipid (PL) and triacylglycerol (TAG)) and fatty acid (20:5n-3 (EPA), 22:6n-3 (DHA) and 18:4n-3 (SDA)) percentage composition (%) and mass (ug) in relation

to sea surface temperature (SST), chlorophyll *a* (Chl *a*) and their interaction terms. Chl *a* was measured at both a Commission for the Conservation for Antarctic Marine Living Resources (CCAMLR) region wide scale (CCAMLR region) and at an 8-day 3 km x 3 km (8D 3x3) pixel scale. Values given are for: P values, r^2 values (italics) and χ^2 values (bold) for the model of best fit. Cells that are greyed out have a P value < 0.05, an r^2 of >0.5 and a χ^2 value > 0.1.

	SST	Chl *a (CCAMLR)*	Chl *a (8D 3x3)*	SST*Chl *a (CCAMLR)*	SST*Chl *a (8D 3x3)*
TL DM (mg g^{-1})	< 0.001 (*0.168*) **0.081**	0.003 (*0.059*) **0.018**	0.124 (*0.034*) **0.238**	< 0.001 (*0.217*) **0.081**	0.672 (*0.084*) **0.238**
PL %	0.652 (*-0.006*) **0.028**	0.001 (*0.102*) **0.005**	0.001 (*0.245*) **0.251**	0.098 (*0.118*) **0.027**	0.006 (*0.209*) **0.251**
TAG %	0.004 (*0.054*) **0.006**	< 0.001 (*0.121*) **<0.001**	0.731 (*-0.021*) **0.085**	0.049 (*0.164*) **0.007**	0.587 (*-0.041*) **0.085**
EPA %	0.001 (*0.082*) **0.079**	0.001 (*0.078*) **0.017**	0.002 (*0.188*) **0.241**	0.296 (*0.112*) **0.080**	0.021 (*0.157*) **0.241**
EPA (ug)	< 0.001 (*0.122*) **0.239**	0.229 (*0.004*) **0.239**	0.007 (*0.146*) **0.238**	0.082 (*0.184*) **0.239**	0.029 (*0.142*) **0.238**
DHA %	0.001 (*0.092*) **0.081**	0.016 (*0.037*) **0.018**	0.198 (*0.017*) **0.241**	0.426 (*0.102*) **0.081**	0.364 (*-0.010*) **0.241**
DHA (ug)	< 0.001 (*0.123*) **0.239**	0.070 (*0.018*) **0.239**	0.529 (*-0.014*) **0.238**	0.345 (*0.186*) **0.239**	0.594 (*0.026*) **0.238**
SDA %	0.121 (*0.010*) **0.081**	0.152 (*0.008*) **0.018**	0.122 (*0.034*) **0.241**	0.263 (*0.065*) **0.081**	0.042 (*0.126*) **0.241**
SDA (ug)	0.001 (*0.089*) **0.239**	0.057 (*-0.003*) **0.239**	0.417 (*0.057*) **0.238**	0.910 (*0.139*) **0.239**	0.415 (*0.034*) **0.238**
16:0 %	0.007 (*0.045*) **0.079**	0.063 (*0.019*) **0.017**	0.292 (*0.003*) **0.241**	0.997 (*0.032*) **0.080**	0.501 (*-0.035*) **0.241**
16:0 (ug)	< 0.001 (*0.143*) **0.239**	0.095 (*0.014*) **0.239**	0.382 (*-0.005*) **0.238**	0.011 (*0.209*) **0.239**	0.635 (*-0.017*) **0.238**
16:4n-1 %	0.305 (*0.000*) **0.082**	0.819 (*-0.007*) **0.019**	0.045 (*0.073*) **0.241**	0.606 (*-0.021*) **0.083**	< 0.001 (*0.367*) **0.241**
16:4n-1 (ug)	0.051 (*0.021*) **0.239**	0.541 (*-0.005*) **0.239**	0.003 (*0.171*) **0.238**	0.263 (*0.052*) **0.239**	0.001 (*0.280*) **0.238**
16:1n-7c %	0.111 (*0.011*) **0.079**	0.027 (*0.030*) **0.017**	0.496 (*-0.013*) **0.241**	0.415 (*0.024*) **0.080**	0.078 (*0.028*) **0.241**
16:1n-7c (ug)	< 0.001 (*0.115*) **0.239**	0.090 (*0.015*) **0.239**	0.573 (*-0.016*) **0.238**	0.002 (*0.192*) **0.239**	0.266 (*-0.016*) **0.238**
16:1/16:0 ratio (ug)	0.289 (*0.001*) **0.239**	0.072 (*0.017*) **0.239**	0.751 (*-0.022*) **0.238**	0.320 (*0.010*) **0.239**	0.077 (*0.024*) **0.238**
EPA/DHA ratio (ug)	0.686 (*-0.006*) **0.239**	0.482 (*-0.004*) **0.239**	0.001 (*0.271*) **0.238**	0.522 (*-0.016*) **0.239**	0.001 (*0.285*) **0.238**
Phytol %	0.046 (*0.022*) **<0.001**	0.353 (*-0.001*) **<0.001**	0.507 (*-0.013*) **0.001**	0.549 (*0.019*) **<0.001**	0.457 (*-0.001*) **0.001**
Phytol (ug)	0.199 (*0.005*) **<0.001**	0.246 (*0.003*) **<0.001**	0.507 (*-0.013*) **0.001**	0.706 (*0.002*) **<0.001**	0.457 (*-0.001*) **0.001**

Table 4.5: Euphausia superba (collected from South Georgia) total lipid (mg g^{-1}) dry mass (TL DM), lipid class (phospholipid (PL) and triacylglycerol (TAG)) and fatty acid (20:5n-3 (EPA), 22:6n-3 (DHA) and 18:4n-3 (SDA)) percentage composition (%) and mass (ug) in relation to sea surface temperature (SST), chlorophyll (Chl a) and their interaction terms. Chl *a* was measured at both a Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) region wide scale (CCAMLR region) and at an 8-day 3 km x 3 km (8D 3x3) pixel scale. Values given are for: P values, r^2 values (italics) and χ^2 values (bold) for the model of best fit. Cells that are greyed out have a P value < 0.05, an r^2 of >0.5 and a χ^2 value > 0.1.

	SST	Chl *a (CCAMLR)*	Chl *a* (8D 3x3)	SST*Chl *a (CCAMLR)*	SST*Chl *a* (8D 3x3)
TL DM (mg g^{-1})	0.003 (*0.054*) **0.246**	0.219 (*0.008*) **0.240**	0.084 (*0.095*) **0.236**	< 0.001 (*0.299*) **0.240**	0.336 (*0.337*) **0.236**
PL %	0.103 (*0.115*) **0.264**	0.013 (*0.083*) **0.249**	0.019 (*0.195*) **0.236**	0.001 (*0.297*) **0.249**	0.001 (*0.301*) **0.236**
TAG %	0.206 (*0.004*) **0.102**	0.010 (*0.088*) **0.249**	0.083 (*0.095*) **0.241**	0.001 (*0.292*) **0.249**	0.163 (*0.194*) **0.241**
EPA %	0.095 (*0.012*) **0.241**	0.083 (*0.033*) **0.078**	0.001 (*0.419*) **0.236**	0.002 (*0.235*) **0.240**	0.003 (*0.437*) **0.236**
EPA (ug)	0.035 (*0.023*) **0.239**	0.105 (*0.027*) **0.239**	0.178 (*0.041*) **0.236**	0.001 (*0.179*) **0.239**	0.645 (*0.032*) **0.236**
DHA %	0.863 (*-0.007*) **0.241**	0.003 (*0.120*) **0.240**	0.001 (*0.377*) **0.236**	0.001 (*0.280*) **0.240**	<0.001 (*0.611*) **0.236**
DHA (ug)	0.026 (*0.029*) **0.239**	0.602 (*-0.012*) **0.239**	0.052 (*0.128*) **0.236**	0.001 (*0.149*) **0.239**	0.817 (*0.072*) **0.236**
SDA %	0.005 (*0.046*) **0.087**	0.076 (*0.035*) **0.019**	0.594 (*-0.033*) **0.236**	0.583 (*0.121*) **0.085**	0.991 (*0.062*) **0.236**
SDA (ug)	0.007 (*0.042*) **0.239**	0.083 (*-0.010*) **0.239**	0.551 (*0.095*) **0.236**	0.409 (*0.162*) **0.238**	0.029 (*0.269*) **0.236**
16:0 %	0.173 (*0.006*) **0.241**	0. 018 (*0.072*) **0.242**	0.036 (*0.155*) **0.246**	0.022 (*0.163*) **0.242**	0.642 (*0.216*) **0.246**
16:0 (ug)	0.277 (*0.001*) **0.239**	0.008 (*0.095*) **0.239**	0.001 (*0.381*) **0.236**	0.001 (*0.281*) **0.239**	0.001 (*0.497*) **0.236**
16:4n-1 %	< 0.001 (*0.104*) **0.004**	0.236 (*0.007*) **0.004**	0.046 (*0.137*) **0.023**	0.903 (*0.136*) **0.004**	0.314 (*0.122*) **0.023**
16:4n-1 (ug)	0.001 (*0.077*) **0.239**	0.841 (*-0.016*) **0.239**	0.481 (*-0.022*) **0.236**	0.030 (*0.119*) **0.239**	0.472 (*-0.050*) **0.236**
16:1n-7c %	0.756 (*-0.006*) **0.241**	0.001 (*0.103*) **0.239**	0.007 (*0.265*) **0.236**	0.001 (*0.279*) **0.239**	0.956 (*0.247*) **0.236**
16:1n-7c (ug)	0.330 (*-0.000*) **0.239**	0.003 (*0.125*) **0.239**	0.001 (*0.443*) **0.236**	< 0.001 (*0.336*) **0.239**	<0.001 (*0.551*) **0.236**
16:1/16:0 ratio (ug)	0.985 (*-0.007*) **0.239**	0.015 (*0.078*) **0.239**	0.020 (*0.195*) **0.236**	0.004 (*0.225*) **0.239**	0.978 (*0.127*) **0.236**
EPA/DHA ratio (ug)	0.245 (*0.002*) **0.239**	0.004 (*0.114*) **0.239**	0.949 (*-0.047*) **0.236**	0.033 (*0.155*) **0.239**	0.398 (*0.029*) **0.236**
Phytol %	0.252 (*0.002*) **<0.001**	0.306 (*0.001*) **<0.001**	0.859 (*-0.046*) **<0.001**	0.507 (*0.015*) **<0.001**	0.691 (*-0-070*) **<0.001**
Phytol (ug)	0.059 (*0.018*) **<0.001**	0.783 (*-0.015*) **<0.001**	0.482 (*-0.023*) **<0.001**	0.603 (*-0.008*) **<0.001**	0.387 (*-0.013*) **<0.001**

Using environmental parameters as predictors of fatty acid biomarkers

The WAP had the most models that fit environmental factors with a significant relationship to the biomarkers in the krill found in that region (Table 4.3). The SG region had the next highest number of models that fit the biomarker/environment interaction relationship (Table 4.5). Both the overall South Atlantic area and the SOI had models that had less significant effects and correlations between the krill dietary biomarkers and the environment in those regions (Tables 4.2 and 4.4). Based on these simple models, the best areas (due to their consistency and predictability in environmental factors) for use to examine krill dietary biomarkers are the WAP and SG, and the best environmental predictors for krill diet are SST, Chl *a* (8D 3x3) concentrations and the interaction of SST and Chl *a* (CCAMLR) concentrations (Tables 4.2-4.5).

The inter-relationships between environmental factors showed that TL DM increased as SST decreased in summer and autumn, but TL DM decreased as did SST in winter and spring. This was independent of Chl *a* levels, which were highest in the SOI in 2014, but the highest TL DM levels were in the WAP in 2016 (Figure 4.6). The percentage of EPA increased after a decrease in Chl *a* levels (grazing effect), but generally followed the same yearly trends as Chl *a*. EPA was high then decreased in 2014, plateaued in 2015 and again started high and decreased throughout the summer and autumn of 2016 (Figure 4.6).

In terms of the fit of models, no models had an adjusted r^2 value greater than 0.5 for the pooled data of the entire South Atlantic region (Table 4.2). The CCAMLR region data had multiple models with good fit with many models exceeding an adjusted r^2 of 0.5. DHA percentage and 16:1n-7c mass fitted well with the interaction between SST and Chl *a* (8D 3x3) (r^2 = 0.611 and 0.551, respectively) in SG (Table 4.5). Whilst no fatty acids had an r^2 above 0.5 at the SOI (Table 4.4). DHA percentage was close to fitting with Chl *a* (8D 3x3) with an r^2 value of 0.498 at the WAP (Table 4.3). SST and Chl *a* (CCAMLR) fitted to PL and

TAG percentages in the WAP (r^2: 0.564 and 0.559, respectively). EPA percentage fitted well with an adjusted r^2 of 0.559 for its WAP model of SST and Chl *a* (CCAMLR).

Better fits were found, however, by using the SST and Chl *a* (8D 3x3) interaction in the model at the WAP (Table 4.3). TL DM, PL and TAG percentages fitted this interaction with adjusted r^2 values of 0.610, 0.655 and 0.568, respectively. EPA percentage (r^2: 0.689) and EPA, DHA and 16:1n7c masses (r^2: 0.615, 0.892, 0.621) all fitted the SST and Chl *a* (8D 3x3) interaction at the WAP (Table 4.3). Additionally, SDA mass correlated with SST and Chl *a* (8D 3x3) at the WAP (r^2: 0.560; Table 4.3).

The best model fit for all of those tested was DHA mass at the WAP using the SST and Chl *a* (8D 3x3) interaction with an adjusted r^2 value of 0.892. This can be seen in Figure 4.7 which compares the slopes and fit of models from the different CCAMLR sub-areas to DHA mass against SST and Chl *a* (CCAMLR) and the 95% confidence interval around the model.

χ^2 values are shown for all models whether the pooled South Atlantic models (Table 4.2) or for the CCAMLR specific sub-areas (Tables 4.3-4.5).

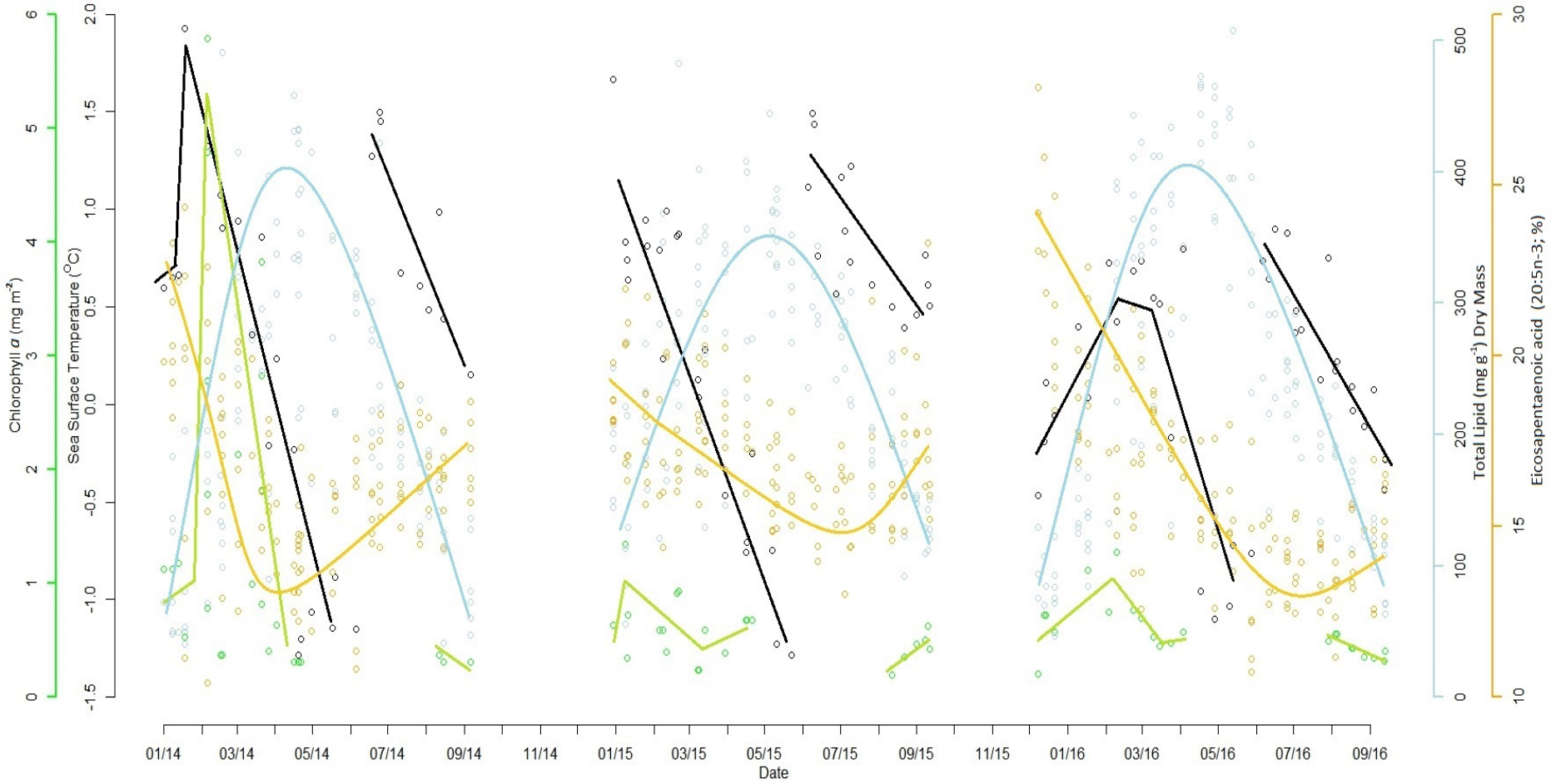

Figure 4.6: Multi Y axis plot of sea surface temperature (°C; black), total lipid (mg g^{-1}) dry mass (blue), chlorophyll *a* levels (mg m^{-2}; green) and eicosapentaenoic acid (20:5n-3) percentage (%; yellow) for dates of krill (*Euphausia superba*) sample collection. Lines drawn for illustrative purposes to show general trends.

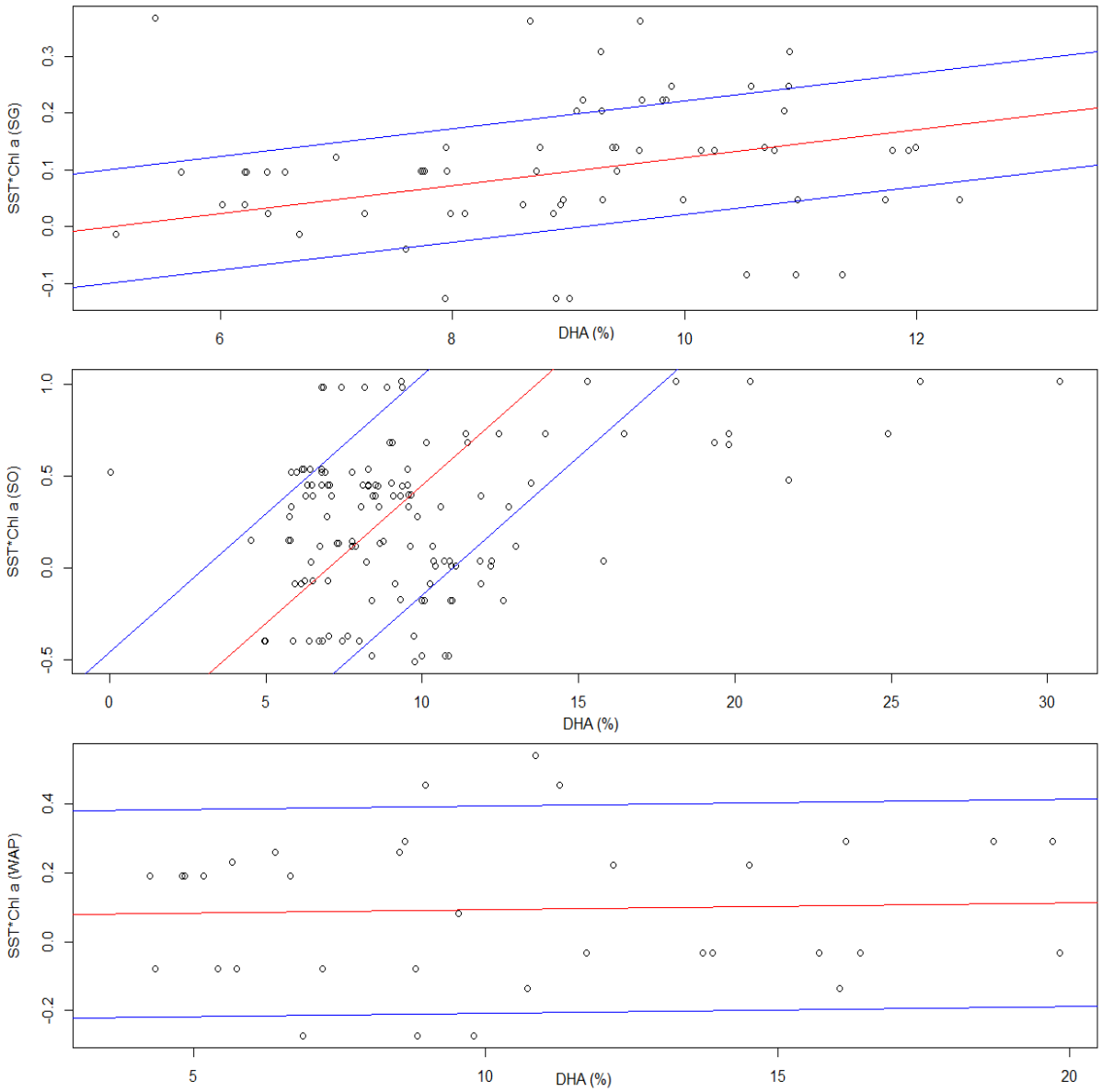

Figure 4.7: Slopes of the models of best fit (red) and the 95% confidence interval for that model (blue) for docosahexaenoic acid (DHA; 22:6n-3) percentage (%) in *Euphausia superba* sampled in the different Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) sub-areas (West Antarctic Peninsula (WAP), South Orkney Islands (SOI) and South Georgia (SG)) against sea surface temperature (°C, SST) and chlorophyll *a* (mg m^2, Chl *a*).

DISCUSSION

Krill lipid content and composition, specifically their fatty acid dietary biomarkers, correlate with changes in broad scale environmental data (SST and Chl *a* levels) derived from satellites.

Krill eat, metabolise, and store lipids and fatty acids derived from their prey throughout the summer and early autumn when waters are warmer (higher SST) with more available food (diatoms and flagellates; higher Chl *a*) (Garibotti et al., 2005, Ericson et al., 2018a, Hellessey et al., 2018). In turn, they use these fatty acid and lipid stores during winter and early spring (lower SST and lower Chl *a*), resulting in a decrease in lipid, fatty acid and therefore n-3 LC-PUFA amounts (Ericson et al., 2018a, Hellessey et al., 2018). This change of fatty acid composition causes an increase in their other fatty acid composition percentages, although the fatty acid masses may not change. During summer and autumn, decreases in specific fatty acid percentages (EPA, DHA, 16:0 and phytanic acid (derived from phytol, a side chain of chlorophyll)), follow the decrease of SST. This is predominantly due to the overall increase in krill TL DM shifting the fatty acid composition in these seasons to lipids used more for reproduction and over winter survival (Hagen et al., 1996, Ju and Harvey, 2004, Yoshida, 2009, Schmidt et al., 2014, Kawaguchi, 2016). Similarly, masses of EPA, DHA, 16:0, 16:4n-1, 16:1n-7c and phytanic acid increased through summer and autumn as krill laid down lipid stores for eggs, mostly n-3 LC-PUFA, and increased their TAG percentages prior to winter. This increase in fatty acid mass had an inverse relationship to SST in summer and autumn and may be due to a grazing effect and/or the lag effect of lipids being metabolised after the spring/summer algal bloom (Schmidt et al., 2012, Behrenfeld et al., 2017).

The large increase in Chl *a* seen during the summer of 2014 in the SOI was strongly correlated to DHA mass and percentage for that season and year. This suggests that krill were predominantly eating flagellates in the summer of 2014 near the SOI and that this flagellate

bloom was detected as extremely elevated green ocean colour and hence Chl *a* levels at the time. Being able to detect the bloom on the same day and at the same location of krill harvest was purely coincidental. Environmental conditions such as sun angle, cloud cover and sea ice did not interfere with ocean colour data capture for that location over that period of time, allowing for one of the best coincidental match ups of environmental data and krill fatty acids throughout the sampling period.

The decrease of SST at SG during winter and spring, however, had an inversely proportional relationship with the increase of these same krill fatty acid percentages (EPA, DHA, 16:0 and phytanic acid). The fatty acid masses in krill decreased in proportion to the decrease in SST at SG. It is thought that this may be related to krill using their lipid stores over winter and spring, a time when SST is lower and Chl *a* in the open ocean is lower (Schaafsma et al., 2017, Kohlbach et al., 2018), and most algae is bound in sea ice (Schmidt et al., 2014, Kohlbach et al., 2017, Meyer et al., 2017, Schaafsma et al., 2017). However, sea ice is not as prevalent at SG during winter as it is at the WAP and SOI, so herbivorous fatty acid sources would be lacking. The 18:1n-9c/18:1n-7c ratio has been previously used to show that krill move from a more herbivorous diet in the summer/autumn to a more omnivorous diet in winter and spring (Mayzaud et al., 1998, Schaafsma et al., 2017, Ericson et al., 2018a). This dietary shift would also be seen as a decrease of fatty acid masses from herbivorous sources (e.g. diatoms and flagellates), however, n-3 LC-PUFA are preferentially conserved in krill as they serve as a major fatty acid functional group for krill health and growth (Ju and Harvey, 2004, Alonzo et al., 2005, O'Brien et al., 2011, Virtue et al., 2016). Therefore, decreases in 16:0, 16:4n-1, 16:1n-7c and phytanic acid masses would be much larger than decreases in EPA and DHA masses, causing their total fatty acid composition percentages to increase proportionally and inversely to SST at this time of year.

Chl *a* levels, indicated from extremely green ocean colour data, could be derived from diatom blooms (Moore and Abbott, 2002, Johnson et al., 2013, Zeng et al., 2016). These blooms may also be from flagellates which are seen as high green ocean colour values too (Moore and Abbott et al., 2002, Zeng et al., 2016), such as those seen at the SOI in the summer of 2014. Chl *a* (8D 3x3) levels correlated positively with EPA percentage, and Chl *a* (overall) levels positively related to phytanic acid percentage and mass. However, no flagellate lipid biomarkers in krill were significantly related to Chl *a* data overall, so it is more likely to come from diatom sources, which would then be seen in diatom markers such as EPA, phytanic acid and a high 16:1/16:0 ratio. Diatoms are likely the major source of phytol-derived phytanic acid in this case, as EPA is a dominant FA in diatoms and high 16:1/16:0 and EPA:DHA ratios were observed, reflecting greater diatom abundance (Morris et al., 1984, Mayzaud et al., 1998, Schaafsma et al., 2017, Ericson et al., 2018a). Other diatom markers weren't as high, possibly due to the differing rates of fatty acid metabolism (Huntley et al., 1994, Mayzaud et al., 2000, Hagen et al., 2001). Biomarkers with faster uptake rates, seen in larger quantities both in the krill's diet and in lipid storage, would be faster to track and could fluctuate more closely to what is seen in the local environment (e.g. small scale blooms and an increase in Chl *a*). EPA and phytanic acid are both readily absorbed and metabolised by krill and can therefore track Chl *a* levels in the environment more closely immediately after a bloom event. EPA can be readily absorbed and stored in the PL of krill. However, some EPA is present (at low levels) within the TAG of krill as well and this may be metabolised even faster than the EPA stored in PL, as it does not need to be converted to PL from the primary dietary source (Ericson et al., 2019b).

Fatty acid biomarker percentages associate better with the Chl *a* (overall) data, however, fatty acid masses are more highly associated with the more specific and localised Chl *a* (8D 3x3) data. Krill maintain their percentages of fatty acids between years, seasons and locations

(Ericson et al., 2018a), so large scale Chl *a* data might not show smaller fluctuations. Krill fatty acid masses can change dramatically (up to a 10 fold increase between summer and early winter, Ericson et al., 2018a), and hence Chl *a* (8D 3x3) data fluctuations are more apparent in localised areas, but not in the overall Chl *a* data. The interaction between SST and Chl *a* (overall) showed a significant relationship to EPA, SDA and 16:0 percentages, while EPA, DHA, and 16:1n-7c masses were more significantly related to the SST and Chl *a* (8D 3x3) interaction. Therefore, using different scales of Chl *a* in the SST and Chl *a* interaction within the models can provide a better prediction for either krill fatty acid percentages or masses, depending on the scale of Chl *a* pixel used. Reasons for inconsistencies may be due to differences between regions as these vary with the local environment in that area. These environmental differences will influence the primary production and hence diet of krill in these regions. Therefore, the biomarkers will vary between regions naturally, but may still correlate with the environmental data from that region (e.g. SST and Chl *a* from WAP will correlate with biomarkers from krill within the WAP but not from SOI). Many of the major essential krill fatty acid biomarkers were correlated to SST, Chl *a* and their interaction terms at varying scales.

Tukey tests revealed the interaction with TL DM was mainly driven by SST, and not Chl *a*, and that SST had a close relationship to TL DM in all locations. Because this relationship holds without the Chl *a* interaction term, it can be assumed that SST drives TL DM levels more than Chl *a* levels do. TL DM relates well to SST and Chl *a* (8D 3x3) interactions as it is scaled to the krill's weight, which may be affected by both temperature (Atkinson et al., 2006) and its stomach and digestive gland weighing more from being full from chlorophyll rich items (Morris et al., 1984, Virtue et al., 1993a, Alonso et al., 2005). As TL DM naturally increases throughout summer and autumn and decreases throughout winter and spring (Hellessey et al., 2018), this would also coincidentally inversely follow the decrease of SST

in summer and autumn, and decrease proportionally to SST in winter and spring. Therefore, the relationship between SST and TL DM may be coincidental due to the seasonal shift in how krill use their lipids aligning with seasonal shifts in SST. At the regional scale, TL DM was strongly related to Chl *a* (CCAMLR) levels in the SOI and WAP, although not in SG. This may be due to krill having a more herbivorous diet during summer and autumn when krill are harvested from the SOI and WAP and a more omnivorous diet during winter and spring whilst they are harvested at SG.

The interaction between SST and Chl *a* (CCAMLR) was significant for SG, however, and also the WAP, but not for the SOI. This may be due to larger SST shifts at the more extreme ends of the latitudinal scale at WAP and SG (Rayner, 2003, Murphy et al., 2007). These locations would have the greatest extremes in environment, particularly for SST (Morris et al., 1984, Rayner, 2003), and as such any variations may explain why these locations show a strong relationship between TL DM and the environment. Similarly, at SG, Chl *a* (CCAMLR) was consistently low for all of winter and spring (when able to be recorded), whereas TL DM decreased dramatically during this period, so this may be giving false model fits for this area at this time of year. If readings of Chl *a* levels were possible throughout the winter season, a more closely related trend might be seen.

As TAG is a storage lipid in krill (Hagen et al., 1996, Hellessey et al., 2018), it decreases from late summer though to the following late spring, when krill build stores for both reproduction (Varpe et al., 2007, Kawaguchi, 2016) and survival over winter (Hagen et al., 1996, Ju and Harvey, 2004, Schmidt et al., 2014, Kohlbach et al., 2018). This peak in TAG also follows the peak in summer algal blooms (and thus peak Chl *a*) and is seen as a lag effect. This lag is due to both the time it takes for krill to metabolise TAG and the rate that krill convert fatty acids in algal TAG to their own PL stores. The PL percentages in krill tracked with Chl *a* (overall and 8D 3x3). PL has also been reported as a storage lipid in krill

(Hagen et al., 1996), and is known to be vital for the storage of essential n-3 LC-PUFA for reproduction (Yoshida, 2009, Ross and Quetin, 2000, Varpe et al., 2007, Schmidt et al., 2012, and krill health (Mayzaud et al., 1997, Yoshida et al., 2011). Interestingly, PL levels were not related to changes in SST, but tracked well with changes in Chl *a*, possibly due to the way that polar phytoplankton blooms can occur via a boom-bust cycle (Behrenfeld et al., 2017). Many types of algae in polar latitudes are high in PL (Nichols et al., 1986, Nichols et al., 1988, Skerratt et al., 1995, Kohlbach et al., 2015), and are also very green in colour, which could be one reason why these are so closely related to Chl *a* levels. PL can be incorporated very quickly into krill tissue as krill predominantly store their lipids as PL (Hagen et al., 1996). Therefore, there would be little lag between ingestion, metabolism and incorporation. Our data shows there is little to no lag seen between Chl *a* data and PL percentages.

The EPA:DHA ratio in krill was significantly related to Chl *a* (8D 3x3). A higher EPA:DHA ratio suggests that krill are consuming more diatoms than flagellates in their diet (Ericson et al., 2018a and 2018b). This relationship could be due to diatom and flagellate blooms affecting the ocean colour readings (higher green values) from satellite more so than other factors (Moore and Abbott, 2002, Johnson et al., 2013, Kahru et al., 2017). The green colour can be detected relatively easily in the ocean (IOCCG, 2015) and ocean colour is changing far faster with the climate than was predicted by models (Dutkiewicz et al., 2019). These changes in plankton community assemblages could also be changing faster than expected (Deppeler and Davidson, 2017, Hancock et al., 2018), which would be reflected in a changing EPA:DHA ratio. Krill potentially prefer a more diatom-based diet when blooms occur, even if flagellates are available in the water column (Behrenfeld et al., 2017, Deppeler and Davidson, 2017, Kohlbach et al., 2018). Such a dietary preference might skew the EPA:DHA ratio of krill, and could be related to the amount of Chl *a* being detected via remote sensing (Behrenfeld et al., 2017), whether at the 8D 3x3 or overall scale. The

16:1/16:0 ratio shows differences in plankton types being consumed by krill (Mayzaud et al., 1998, Ericson et al., 2018a), and was not associated with any Chl *a* or SST data over the whole South Atlantic but did show differences at smaller CCAMLR region scales. This may be due to krill diet shifting with seasons at the same time as the krill fishery also shifts it's fishing location at the end of autumn (from SOI or WAP) to the start of winter (SG).

The krill fishery operates at SG during winter and spring when algal populations are naturally lower in the water column (Schaafsmas et al., 2017, Kohlbach et al., 2018), and most algae is bound in sea ice (Schmidt et al., 2014, Kohlbach et al., 2017, Meyer et al., 2017, Schaafsma et al., 2017). Remote sensing during winter in polar regions is particularly hard for multiple reasons e.g. cloud cover, sea ice, and sun angle (IOCCG, 2015). These difficulties create gaps in the Chl *a* data available at SG, which may be falsely lowering these levels. Ground truthing the Chl *a* concentrations in SG throughout the winter season is one way of confirming this remote sampling data bias. Large-scale and long-term studies such as the Palmer Long-Term Ecosystem Research (LTER) program (Holm-Hansen et al., 1994, Helbling et al., 1995, Smith et al., 1995, Moline et al., 1997) and the U.S. Antarctic Marine Living Resources (AMLR) program (Helbling et al., 1995, Phleger et al., 2002) can ground truth their Chl *a* recordings by being present year round in a location to take water samples for Chl *a* analysis. Future technological advancements may also assist with such ground-truthing, including the use of deployed moorings that take water samples and record algal fluorescence year-round. Improving satellite algorithms for ocean colour data to be converted into Chl *a* concentrations in polar regions would significantly increase the number of data points available throughout winter. In turn, such enhanced data could then be better related to other factors, such as krill diet, with the remote sensed Chl *a* data being closer to the true Chl *a* levels present at that time of year.

Additionally, due to krill swarming the spatial and temporal scales used may not be the tightest to fit krill dynamics as more than a single krill aggregation may be present in a 3 km x 3 km grid, and krill diet may vary greatly over an 8-day period. However, total lipid content varies slowly, particularly in the whole animal, as does their fatty acid profile. So, large dietary differences in krill aren't expected to be seen at this level over the period of 8 days or at a scale of 3 km x 3 km. If, however, this analysis was using the lipids and fatty acids of krill stomachs or digestive glands, then this may have a more significant impact as these would change greatly over the period of 8 days and would show variation within a 3 km x 3 km grid.

This study used satellite-derived ocean colour and SST data in conjunction with fatty acid content and composition in krill diet at different times of year and in different locations. Cross-disciplinary work such as in this study is promising as it enables remote sensing and satellite oceanography specialists to better link with biological, physiological and ecological specialists. This collaboration may enable issues such as winter sampling of Chl *a* through satellites to be more well understood and solutions to issues such as sea ice and cloud cover to be resolved at a scale that is meaningful to the biology associated to that Chl *a* reading, whether primary producers or krill.

The relationship between SST, Chl *a* and krill lipid biochemistry presented here could be expanded to examine similar relationships in krill diet and krill lipid content and composition in other regions around Antarctica. The approach could also be used for other marine-based species both in the Antarctic and other polar areas where sampling is restricted.

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Chapter 5: General Discussion

During this project I studied the within and between seasonal variation in the lipid and fatty acid biochemistry of krill. Such data was used to investigate temporal variation in the diet of adult krill. Krill diet was inferred from the analysis of a unique set of fishery-derived samples. The samples were collected over large spatial and temporal scales that I then related to remotely-sensed environmental data. These approaches have provided new insights into how krill diet and lipid content vary throughout and between seasons, years, geographical areas and with the environment.

The three main outcomes of this research were:

- A detailed interannual and seasonal examination of the cycle in krill total lipid content and individual lipid class composition and content. These cycles were observed over a near-continuous three-year time period in the South Atlantic. This aspect of the study resulted from the availability of high-resolution fishery-derived samples;
- Strong regional differences in krill diet were observed during the late-summer spawning period. These differences were detected from analysis of the fatty acids and sterols from the neutral lipid fraction in both sexes of krill. Results were further supported by similar analyses performed for their main digestive organs (digestive gland and stomach);
- Krill lipid dynamics, including their fatty acid biomarkers, were related to the sea surface temperature (SST) and chlorophyll *a* (Chl *a*) concentration derived from remotely-sensed satellite data.

Results from my research could be of use in developing and better parameterising ecosystem and energy budget models. Utilising the links between the environment (SST and Chl *a*) and krill fatty acids will directly improve ecosystem models relating krill physiology and diet to their environment. Similarly, understanding krill lipid dynamics at a seasonal, regional, and

interannual scale will assist in improving krill energy budgets and life history models, as lipids are the key energy molecule in krill used to predict their condition and recruitment potential. These results will improve the understanding of seasonality of krill biology, and also assist in predicting krill recruitment and dietary changes under changing climate scenarios.

Seasonality in krill lipid content and composition

The seasonal and interannual trend in krill lipid content and composition was detailed by utilising high-resolution fishery-derived samples. Krill total lipid content showed a temporally sinusoidal pattern, previously surmised, but not quantified or detailed at such a high resolution as presented here. This clear seasonal and interannual trend in krill total lipid content was also seen in their component lipid classes. The fluctuations in krill lipid classes, specifically TAG, had long been assumed to be used for lipid storage, although this had not been unequivocally demonstrated due to the lack of high-resolution winter sampling of krill (Marschall, 1988, Huntley et al., 1994, Hagen et al., 1996, Atkinson et al., 2002, Ju and Harvey, 2004, O'Brien et al., 2011, Schaafsma et al., 2017). By utilising fishery-derived samples, this issue of winter sampling for TAG levels has now been further resolved. The significance of TAG as a storage lipid, alongside PL as Hagen et al. (1996) has shown, will require further investigation as to how and why krill metabolise TAG through the seasons and if this varies at different geographical scales.

The seasonal differences observed in the major lipid classes reflected their differing roles in key physiological and biochemical processes: growth, storage, and reproduction. The seasonal relative levels of TAG followed the temporal sinusoidal trend seen in total lipid content, with a peak in autumn and lows in spring. This corresponds to TAG being used as a storage lipid for energy reserves over the winter months, with little other use by krill unlike for PL, which can be both a structural and storage lipid class in krill. PL relative levels in krill also showed a seasonal response, but the seasonal peaks were out of phase with highs occurring in

spring and lows in autumn. The high PL levels seen in these seasons could be due to its primary uses; for growth during the spring and summer when conditions are optimal, as well as for energy and lipid transfer during the reproductive season, particularly in gravid females. However, gravid female krill contain much higher levels of TAG than spent females because krill eggs are high in TAG (Marschall and Hirche, 1984, Tarling et al., 2009b). Gravid females have almost no available space for storing polar lipids due to the available carapace space being used for egg storage and production instead. The late-summer spawning period is of particular interest, as the ability of krill to reproduce is also linked to their ability to store enough lipids for growth and survival throughout winter (Mayzaud et al., 1998, Atkinson et al., 2006, Schmidt et al., 2012). Observed TAG levels in female krill would thus be dependent on the timing of krill spawning and the spring/summer algal bloom.

This variability in the relationship between PL and TAG stores in krill may be due to differences in the timing and spatial extent of algal blooms that occur after the spring sea ice melt (Skerratt et al., 1995, Janout et al., 2016). Increases in both PL and TAG content and relative (%) composition after algal blooms have been observed for northern krill species; *Meganyctiphanes norvegica* (M. Sars), *Thysanoessa inermis* (Krøyer) and *Thysanoessa raschii* (M. Sars) (Falk-Petersen, 1981, Falk-Petersen et al., 1981). Pond et al. (1995) and Ju and Harvey (2004) have previously reported that lipid accumulation, particularly TAG, is tightly linked to seasonal factors such as the timing of reproduction in Antarctic krill species and the timing of the spring/summer algal bloom, which allows for krill to lay down lipid stores. To further explore this crucial spawning period, the differences in krill diet at a regional scale were investigated during this season.

Regional differences in krill diet

The TL, fatty acid and sterol content and composition of whole krill, as well as their digestive glands and stomachs, were examined for samples obtained from all three of the ocean

basins surrounding Antarctica during the spawning period. By comparing fishery-based samples from the South Atlantic Ocean to scientific samples collected from the Southern Indian and Pacific Oceans (where fishery samples are unavailable), regional differences in krill diet could be seen for the first time.

Early studies reported large differences in total lipid (TL) content between krill sexes (Fricke et al., 1984, Mayzaud et al., 1998). The TL (mg g^{-1}) content in Indian and Pacific Ocean sector female krill decreased from sub-adult females to gravid females, and decreased further to spawned females. This trend was not seen in females in the Atlantic Ocean sector, which all had high TL content (mg g^{-1}). This difference may be due to levels of primary production in the Atlantic Ocean sector being higher and also more consistent year-round than in the Indian and Pacific Ocean sectors (El-Sayed and Weber, 1982, Vernet et al., 2008, Westwood et al., 2010). There is a known abundance and consistency of primary production in the Atlantic Ocean sector (Bodungen et al., 1986, El-Sayed and Weber, 1982, Helbling et al., 1995, Korb et al., 2005, Vernet et al., 2008). The relationship between primary production and krill lipid dynamics is currently unknown, however, primary production is known to fluctuate temporally (e.g. the spring/summer bloom). The timing and type of algal blooms available throughout the spawning period will depend on local factors such as SST, light availability, nutrient levels and salinity. Regional differences in the timing of this bloom would therefore also affect krill diet and spawning in each locality. Few studies have looked at differences in the fatty acid or sterol content and composition between the sexes of krill (Mayzaud et al., 1998, Atkinson et al., 2006, Schmidt et al., 2012), but never at this large of a geographical scale.

In my study, the analysis of the neutral lipid fraction of krill digestive glands and stomachs was undertaken for samples collected in all three oceans basins surrounding Antarctica for the first time. Very few previous studies have used neutral lipid-derived fatty acids in dietary analysis, as the procedure is more complex than total lipid-derived fatty acid

analysis (Cabrol et al., 2019). The analysis of the neutral lipid-derived fatty acids has allowed an in-depth detection of both shorter- and longer-term dietary differences between sexes of krill at a regional scale. My work details the shorter-term diet of krill from neutral lipid fatty acid and sterol profiles in samples from the stomach (days) and digestive gland (days-weeks). Using only the fatty acids from the neutral lipid fractions ensures that any signals from the structural components of the cells of the digestive gland and stomach are not included into the dietary marker profiles (Virtue et al., 1993a, Yoshida et al., 2009, Cabrol et al., 2019). The use of neutral lipid-derived fatty acids therefore enabled a better understanding of krill diet, which was found to vary by region in all of the different tissue samples during the late-summer season. My results showed that during the reproductive season digestive gland and stomach samples from Indian Ocean sector krill had a more diatomaceous and carnivorous based diet than digestive gland and stomach samples from krill in the Pacific and Atlantic Ocean sectors. Similarly, differences seen in the diet of the various sex classes of krill was predominantly due to a regional effect.

Within each region, krill of different sexes showed little variation in their fatty acid profiles, which may be expected as they would have the same dietary material available to them. Krill from the Indian Ocean sector were the exception, where sub-adult females and spent females had significantly different dietary signals. The cause of this dietary difference is unknown, but it is hypothesised that spent females may need to regain lipid stores quickly in preparation for the winter months post-spawn, unlike sub-adult females. Another theory is that sub-adult and spent females are feeding in different locales (e.g. continental shelf, deeper water, warmer waters) within the wider region of the Indian Ocean sector as Nicol et al. (2000b) and results for my own samples have shown. Feeding at different locations therefore may be impacting the dietary signatures for sub-adult and spent female krill.

These analyses have allowed for a comparison of krill diet at different temporal scales (days, weeks and long-term) at a regional level. The differences found between these regions suggested that possible environmental factors could be influencing krill diet at these smaller regional scales. Understanding how the environment at these different regional locations impacts krill diet would assist in linking key environmental factors, such as SST and Chl *a* concentrations (a proxy for primary production), to krill lipid dynamics.

The inclusion of environmental data into this regional data set, for example, may assist in addressing why males and gravid females at Indian Ocean sector site 6 had vastly different diets to krill at other sites within the Indian Ocean sector. However, such a study would need more extensive sampling to be undertaken in this region. The collection of krill from scientific expeditions in both the Pacific and Indian Ocean sectors for this work was opportunistic. The collection of krill at a larger scale in these regions would overcome some of the limitations of having smaller sample sizes; these include the limited comparisons, variables and experiments which are currently able to be done. The trends and generalisations that can be drawn from studies using larger sample sets, particularly when investigating factors such as differences in diet between krill sexes, would be greatly improved. Details of krill diet at a seasonal and interannual scale are also still required at a regional scale.

Relating SST and Chl a to krill diet

To examine these dietary differences further, I used remotely-sensed SST data and Chl *a* concentrations to investigate whether these environmental variables could be related to the long-term krill lipid data obtained from the fishery-derived samples collected in the South Atlantic Ocean. Understanding the dynamics of the seasonal cycles of krill lipid content and composition in relation to the seasonal fluxes in primary production is a well-known, but unquantified link in the Antarctic food web. Through the use of high-resolution krill fishery

samples in conjunction with remotely-sensed satellite environmental data, particularly SST and Chl *a*, these patterns have been established and measured for the first time.

Broad scale environmental data from satellites was collected on simultaneous dates and from the same geographic positions as the krill fishery-derived samples. This allowed for the exploration of the links between the environment at that location and the biochemistry of the krill harvested there. The results show that krill lipid biochemistry, specifically their fatty acid dietary biomarkers, track changes in broad scale environmental data such as SST and Chl *a* concentrations derived from satellites.

Interactions between Chl *a* and SST correlated well with krill fatty acid masses and percentages, although it is noteworthy that TL (mg g^{-1}) dry mass (DM) related well to SST, but not to Chl *a*. This observation may be due to large scale environmental changes occurring between seasons. Seasonal changes such as sea ice expansion and atmospheric temperature drops during autumn and winter drive these large scale community composition changes. Therefore, krill lipid dynamics (and hence TL DM) are affected more by these large scale environmental changes than the small changes in algal community composition that occur throughout these same seasons. This community composition change would be seen more in the fatty acid composition of the krill, although it may not be seen in the TL DM of the krill. This may be due to krill storing lipids throughout summer and autumn, and increasing their TL DM, but not their fatty acids. These increases in krill TL DM are influenced more by the seasonal temperature shift than being due to prey community composition and availability.

This study was limited by the fact that some winter and early spring Chl *a* concentrations were not available because of a lack of ocean colour data. Hence, any relationship between SST, Chl *a* and krill lipids at this time cannot therefore be accurately predicted. However, the interaction between SST and Chl *a*, when examined at a CCAMLR

region scale, did give an initial indication that this relationship with krill lipids might be seen year-round. The interaction between SST and Chl *a* (CCAMLR region) matched well with the TL DM of krill at the more extreme ends of the latitudinal scale (South Georgia and the West Antarctic Peninsula). These locations would have the greatest extremes in environmental conditions, particularly for SST (Morris et al., 1984, Rayner, 2003). Variations in SST and Chl *a* at these locales may assist in explaining why they show a stronger relationship between TL DM and their corresponding environment.

Krill lipid classes also showed relationships that varied with the remotely-sensed data. TAG percentage was related to Chl *a* (8D 3x3) and increased with decreasing SST during summer, but not in autumn. As TAG is the main storage lipid in krill (Hagen et al., 1996, Hagen et al., 2001), it would be related to a decrease in SST as the year progresses towards a time when krill need more stores for both reproduction (Varpe et al., 2007, Kawaguchi, 2016) and survival over winter (Hagen et al., 1996, Ju and Harvey, 2004, Schmidt et al., 2014, Kohlbach et al., 2018). The TAG percentage in krill follows the same sinusoidal trend seen in TL DM throughout the seasons, and as TL DM is highly related to SST, it follows that TAG percentage would have the same relationship to SST as TL DM.

Percentages of PL did not correlate well with SST, as the peaks and troughs occurring for PL levels in krill are at the opposite times of year to TAG. PL percentages in krill did, however, correlate well to Chl *a* concentrations at both the overall and 8D 3x3 scale. This may be due to krill utilising and/or absorbing PL faster from phytoplankton that are already naturally high in PL content. It could also be due to krill metabolising TAG from phytoplankton and converting it to PL, causing it to increase, after an algal bloom occurs. Both scenarios would have an increase in Chl *a* concentration in the water due to the occurrence of an algal bloom, and both would result in higher PL percentages being observed in the krill. Further investigation into the mechanisms (e.g. absorption, utilisation and metabolism) of how PL

levels increase in krill, such as through lipidomics, would help to clarify this relationship with Chl *a*.

Whilst this methodology was novel, it could also be expanded to be used with the regional dietary samples of krill. This expansion would facilitate further insights to the impacts of regional environments on the condition and diet of krill around Antarctica. This would also allow for meaningful regional scale changes to be modelled in different climate scenarios.

At a regional scale, ecosystems will be driven by their local environments more so than the wider environment. Having both environmental and dietary food web data links at a regional scale is vital for understanding changes occurring within the different regions. As an example, my research showed that Chl *a*, when examined at an 8-day (8D) 3 km x 3 km (3x3) pixel scale, correlated positively with EPA percentages (as % of total fatty acids) in krill from the Atlantic Ocean sector in summer and autumn. This relationship between the environment (e.g. Chl *a*) and krill fatty acids is uncertain with regards to krill samples from the Indian Ocean sector, which had a more carnivorous and diatomaceous based diet. Carnivory inputs would not be able to be detected through remote-sensing. Insights into the interaction between the environment and krill diet at a regional scale will assist in understanding how krill predate differently around Antarctica.

This study is the first to link remotely-sensed environmental data to krill fatty acid data and the methodologies used here potentially can be further improved, strengthened, and expanded to other areas, and applied to different krill species or other marine organisms. There is a need for further research and experimentation into the linking of remotely-sensed Chl *a* concentrations at different scales to fatty acid content and composition. Examining different phytoplankton functional types through remote-sensing techniques would be another key area for future research. This can be performed by further analysing the ocean colour data and

separating out the chlorophyll values into specific phytoplankton pigment groups, such as red algae, green algae and brown algae (e.g. coccolithophores, diatoms, dinoflagellates). Ideally, these remote-sensing analyses would be ground-truthed with *in situ* sampling of phytoplankton by high throughput methods such as automated shape recognition assisted flow cytometery. In particular, it would be valuable to relate the variation in the relative abundance of different phytoplankton functional groups throughout the year to the seasonal and interannual trends seen in krill lipid dynamics.

Future research

The results of this study indicate five broad areas for future research that would improve our knowledge of krill diet and lipid dynamics.

Firstly, the high-density sampling that was possible through the availability of krill fishery-derived samples has revealed new insights into the seasonal and temporal cycles in krill lipids. If such sample collection could be broadened to include all three Antarctic ocean basins, this would improve our understanding of krill lipid biochemistry as well as krill biology more generally. One potential way of expanding the number of samples would be to utilise the recent expansion of the krill fishery into the Indian Ocean sector of the Southern Ocean (CCAMLR, 2017). Krill distribution and density are known to differ greatly between basins, and improved sampling might help to clarify the drivers of many krill biochemical and biological parameters. Although many multinational research voyages have attempted to cover vast areas (such as the CCAMLR 2000 and 2019 surveys, BROKE, BROKE-West, K-Axis, and the recent survey of Sub-Area 58.4.1 by the Japanese), these voyages rarely cover more than a single basin, month or season. These larger research voyages also normally target specific locations with little overlap between voyage locations, which in turn does not allow for repetition or interannual effects to be accounted for.

The Chinese krill fishery opened an exploratory fishery in the Indian Ocean sector in 2016 in accordance to the CCAMLR conservation management guidelines and policies (CCAMLR, 2017). The continued presence of a Chinese based krill fishery in the Indian Ocean sector would facilitate the sampling of krill in this region in different seasons and would have a much wider geographical spread than current voyages in this sector that are undertaken by national scientific voyages. If samples were to be collected through a similar methodology as those in the Atlantic Ocean sector, the comparison of samples between regions could also become more consistent.

Currently there are no large-scale biological voyages planned for the Pacific Ocean sector, whether by multinational efforts of the scientific community or by industry. Few large-scale expeditions have concentrated on the biology of the Pacific Ocean sector (CCAMLR Area 88) (Bottino, 1974, Mackey et al., 2012, Leonori et al., 2017), although the physical oceanography of the Ross Sea has been studied numerous times (Jacobs et al., 1970, Orsi and Wiederwohl, 2009, Smith Jr et al., 2014). Since the Discovery voyages in the 1820's, only the Antarctic Circumpolar Expedition (ACE) voyage in 2017 has collected biological samples at a large scale in this area, so this region in particular warrants further investigation.

Another way of accessing krill samples from these areas would be to utilise ships of opportunity, such as other fishing vessels, tourist vessels, resupply vessels, and other national scientific voyages. This would increase the number of samples of krill within these regions with minimal effort. Adoption of an agreed set of collection and analysis protocols would ensure that comparable sets of samples were obtained, making additional sample contributions invaluable.

Secondly, this study focussed only on the diet of adult krill as they are numerous within the fishery samples and found year-round at all spatiotemporal scales. Future studies should

aim to examine the diet of larval and juvenile krill, which is known to be substantially different (Atkinson et al., 2002, Virtue et al., 2016, Schaafsma et al., 2017). Further investigations at varying spatial and temporal scales would assist in understanding dietary differences between larval and juvenile krill (and adults), and how their diet may impact development, reproduction and survival.

The climate of the Southern Ocean is changing with waters warming, becoming fresher, and increasing in acidity, the consequences of which on adult krill are still mostly unknown (Kawaguchi et al., 2011, Flores et al., 2012, Bijma et al., 2013, Hill et al., 2013, Kawaguchi et al., 2013, Constable et al., 2014, Barnes and Tarling, 2017, Ericson et al., 2018b, Atkinson et al., 2019). The flow on effects of such changes on larval and juvenile krill diet quality, due to a decrease in sea ice extent and thickness with climate change, warrants further study. A reduction in sea ice will likely impact future krill recruitment and population levels because these life stages require sea ice for food, shelter and protection (Wiedenmann et al., 2009, Massom and Stammerjohn, 2010, Schmidt et al., 2014, Kohlbach et al., 2017, Schaafsma et al., 2017, Kohlbach et al., 2018). Research on the impacts of ocean acidification on adult krill by Ericson et al. (2018b) has included the development and use of an aquarium-based methodology that would be ideal to look into larval and juvenile krill diet, health, development and mortality in relation to ocean acidification also. The link between increased sea ice extent and thickness with increased growth, health and recruitment of juvenile krill is already well understood (Wiedenmann et al., 2009, Schmidt et al., 2014, Schaafsma et al., 2017, Mori et al., 2019). The use of remotely-sensed data for determining sea ice expansion and contraction rates, thickness and extent throughout the seasons could also be useful for enhancing the understanding of how juvenile krill diet and health will change at a larger scale in real time.

If a robust relationship between remotely-sensed sea ice data and larval and juvenile krill lipid dynamics can be determined, then a model of sea ice expansion and contraction with

krill recruitment levels could be developed. Such a model could operate using near real time data which would allow for annual predictions and detection of interannual and long-term trends of krill recruitment. As sea ice contracts southward and thins with climate change, having faster predictions of potential negative effects on the ecosystem by human influences (such as the fishery) will be required to stop additional stress being added to the system. A predictable relationship between remotely-sensed sea ice data and krill recruitment would allow CCAMLR to implement within season fishing regulations as envisaged in the various proposals for feedback management (Constable et al., 2000, Constable and Nicol, 2002, Hill and Cannon, 2013, Hill et al., 2016). Similarly, Chl *a* concentrations and SST could be used for providing adult krill condition and reproduction estimates leading into the spawning season allowing for tighter within season fishery management.

In addition, investigations at varying spatial and temporal scales would assist in understanding the influence of diet on larval and juvenile krill development and maturity. If larval and juvenile krill diet shifts with climate change, due to the assemblages of microbes and primary producers changing (Deppeler and Davidson, 2017, Hancock et al., 2018), this may have direct impacts on larval and juvenile krill growing to maturity and their ability to reproduce. Knowing how larval and juvenile krill diet varies currently at different scales is of benefit to understanding and predicting krill recruitment and survival both now and into the future. Studies on larval and juvenile krill lipid dynamics are highly recommended, particularly if they are able to be coupled with large spatial, temporal or environmental data studies such as presented here, or via the use of aquarium-based studies looking at future ocean scenarios as in Ericson et al. (2018b).

Thirdly, this data adds to and expands upon the work of the Southern Ocean Diet and Energetics Database (SO-Diet; Raymond et al., 2011). This database is collecting dietary analyses from all Southern Ocean species, both historical data and more recently analysed, and

from a variety of different dietary methodologies including stable isotopes, gut content analysis, lipid and fatty acid analysis and DNA analysis.

The inclusion of a large spatial and temporal range of adult krill diets to this database, particularly in the form of lipid and fatty acid data, will inform future studies linking species, or looking at demographics within krill diet to be enhanced. By combining this data with other krill diet and energetic studies a more complete and robust energy budget for adult krill will also be possible. This data can also be used as a baseline, especially within the Atlantic Sector where the data is richest, for future climate studies in relation to adult krill diet or the impact of the fishery on krill diet. Looking for longer term trends in adult krill diet is nearly impossible without combining data from multiple studies, regions, expeditions and nations, therefore the addition of this large and diverse dataset will be extremely useful of larger, longer term trend studies also.

Fourth, my research has demonstrated the utility of using remote-sensing for addressing potential ecological problems linked to krill. A new generation of multinational controlled polar orbiting satellites has recently been launched. The first new satellite, Joint Polar Satellite System-1 (JPSS-1) was launched in November 2017. JPSS-1 includes instrumentation such as the Advanced Very High Resolution Radiometer (AVHRR) and the Advanced TIROS Operational Vertical Sounder (ATOVS). The joint AVHRR/ATOVS on the JPSS-1 will provide data in the visible, infrared and microwave ranges, allowing for a multitude of applications. The JPSS fleet will eventually include five satellites and, if they are calibrated and utilised correctly, such a system could be extremely useful for gaining biologically relevant environmental data, specifically from this hard to access area of the world. Additional ocean colour and biological data from the JPSS would improve future Chl *a* algorithms for the Southern Ocean, provide more winter and early spring ocean colour samples, and provide a platform for other future biological measurements to be undertaken within this polar region.

Finally, there is a need for future biochemical studies into the lipidomics and pigment signatures of krill. Lipidomics is the large-scale study of pathways and networks of cellular lipids in biological systems (Wenk, 2005). Research into krill lipidomics would allow for more detailed answers on how lipids are converted and metabolised within the krill's biological system. Most krill lipidomic studies are focused on the metabolic fate, including a number of biologically active intermediates, of n-3 LC-PUFA when ingested as krill oil in humans (Wenk, 2005, Backes et al., 2014, Méndez et al., 2017, Sung et al., 2019). Currently, studies on krill lipidomics within the krill themselves are rare (Chen, 2012). Knowledge of the molecular conversion of one lipid into another, through a chain of complex reactions, within the cells of krill could potentially be harnessed for commercial purposes. Lipidomic studies could ascertain where and why krill store their lipids based on external influences, such as their environment; or they could find the most efficient way of converting krill dietary inputs into commercially viable n-3 LC-PUFA. Lipidomics is an expanding area of study and the implications for application of this approach at a commercial scale are not yet fully understood, but there is great potential for its use into the future.

Astaxanthin, a red pigment found in krill, already has its own unique market due to its antioxidant qualities and ability to colour the flesh of higher predators (particularly salmon). How and why astaxanthin is metabolised and utilised by krill is still largely unknown (Takaichi et al., 2003). Knowledge of how lipids and their related pigments are converted, utilised and metabolised in krill is an obvious next step for gaining further understanding of krill lipid dynamics. Knowledge of how pigmentation in krill is related to their diet and lipids would be of huge benefit to the krill fishery in their production of krill oil for nutraceutical products, which contain this natural antioxidant (Tou et al., 2007, Barros et al., 2014). Studies and research such as this would be able to provide valuable insights to the fishery on many potential krill based products such as (but not limited to) astaxanthin, chitin and krill enzymes. Studies

into other commercially relevant biochemicals in krill would benefit from also having spatial, temporal and environmental data linked to the wider krill fishery and it's sustainability.

Conclusions

By utilising the spatial and temporal aspects of krill lipid data, fishery decisions can be made based on where and when n-3 LC-PUFA or TL content is highest. Knowing when and where krill lipid content is optimal will facilitate maximising lipid yield whilst minimising krill harvest. This will assist the fishery as the industry, in theory, will not require as much krill to be caught if the lipid content within each krill is greater on average. Similarly, using the environmental links as shown in my research, the fishery will be better able to predict krill lipid and n-3 LC-PUFA content in years with lower/higher Chl *a*, or SST, or both. This will also assist in sustainable fishery management into the future as the environment becomes more unpredictable.

The research undertaken in this thesis has increased the understanding of krill lipid dynamics, which has implications for enhancing knowledge on krill diet and the factors influencing krill diet at different spatial, temporal and environmental scales. The interannual and seasonal trends in krill lipid content and lipid class composition were further resolved with the availability of fishery-derived samples. Regional differences in krill diet were found by examination of the neutral lipid fraction fatty acids of krill digestive glands and stomachs. Krill diet varied with sex at a regional scale during the late-summer spawning period. Krill lipid dynamics, including the composition of key fatty acid biomarkers, were able to be related to satellite-derived Chl *a* concentrations and SST data. The availability of high resolution data on krill lipid dynamics under current environmental conditions will facilitate an improved understanding on krill growth, health, reproduction and predictions of recruitment. Such information will improve both ecosystem and energy budget models, which in turn may lead

to better krill fishery management models allowing for maximum lipid yields whilst potentially minimising krill catch.

Appendices

APPENDIX 1

Supplementary Table 3.1: Composition (as % mean ± SD of total lipids) of lipid classes for each *Euphausia superba* sample type and Southern Ocean sector (Atlantic, Indian and Pacific). Mass for the stomach and digestive gland samples are for the whole sample, not on a per krill basis. (HC - hydrocarbons (including wax and sterol esters); TAG - triacylglycerols; FFA - free fatty acids; ST - sterols; DAG - diacylglycerols; PL - phospholipids; Unknown).

Sample Type	Sector	HC	TAG	FFA	ST	DAG	PL	Unknown
Whole Krill	Atlantic	0.5 ± 0.4	42.6 ± 5.3	3.2 ± 1.7	2.5 ± 3.6	5.8 ± 6.9	43.7 ± 8.2	1.6 ± 2.8
	Indian	0.4 ± 0.7	37.6 ± 10.7	0.1 ± 0.1	2.3 ± 1.8	3.4 ± 10.5	55.0 ± 8.9	1.1 ± 4.4
	Pacific	0.2 ± 0.1	49.1 ± 4.2	1.7 ± 3.5	1.8 ± 1.1	1.2 ± 1.1	45.9 ± 3.6	0.0 ± 0.0
Stomach	Atlantic	1.0 ± 1.4	28.3 ± 5.1	2.2 ± 0.7	1.4 ± 1.3	0.4 ± 0.4	66.6 ± 4.9	0.0 ± 0.0
	Indian	0.0 ± 0.0	33.5 ± 29.7	17.4 ± 15.5	1.3 ± 2.3	1.8 ± 3.5	46.0 ± 22.2	0.1 ± 0.4
	Pacific	0.2 ± 0.3	9.1 ± 10.3	46.5 ± 27.3	0.8 ± 0.1	0.2 ± 0.3	43.0 ± 16.4	0.2 ± 0.3
Digestive gland	Atlantic	1.2 ± 0.9	36.3 ± 6.6	6.8 ± 4.3	0.9 ± 1.4	2.3 ± 3.9	52.5 ± 5.9	0.0 ± 0.0
	Indian	8.7 ± 15.0	57.9 ± 19.1	2.9 ± 5.6	0.8 ± 3.6	0.0 ± 0.0	29.4 ± 8.5	0.0 ± 0.0
	Pacific	0.2 ± 0.3	33.7 ± 3.9	12.5 ± 7.6	0.7 ± 0.3	0.3 ± 0.2	52.5 ± 5.7	0.2± 0.3

Supplementary Table 3.2: *Euphausia superba* fatty acid groups (as % of the total fatty acids; mean ± SD) and selected major dietary fatty acid markers in different Southern Ocean sectors and tissue types (MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, SFA: saturated fatty acids, MSI: marine snow indicator [Σ C_{15}, C_{17} and C_{19} isomers], Copepods: [Σ 20:1n-9c + 22:1n-9c], Carnivory ratio: [18:1n-9c/18:1n-7c], Diatoms: [Σ 16:1n-7c +16:4n-1], CvD: Copepods vs Diatoms ratio [Σ 20:1n-9c + 22:1n-9c/ 16:4n-1]).

Sample Type	Sector	MUFA	PUFA	SFA	MSI	Copepods	Carnivory	Diatoms	CvD	Phytol
Whole Krill	Atlantic	33.9 ± 2.3	33.9 ± 3.4	32.1 ± 1.6	0.7 ± 0.2	1.9 ± 0.5	1.9 ± 0.2	8.4 ± 0.9	2.4 ± 0.8	2.6 ± 1.6
	Indian	40.0 ± 4.1	26.5 ± 6.6	33.8 ± 3.2	0.6 ± 0.3	1.5 ± 0.4	1.7 ± 0.2	8.9 ± 2.1	3.3 ± 1.6	2.8 ± 1.2
	Pacific	31.8 ± 1.5	35.0 ± 2.3	33.2 ± 1.8	0.9 ± 0.1	1.5 ± 0.3	2.3 ± 0.3	6.3 ± 0.6	2.6 ± 0.8	1.9 ± 0.7
Stomach	Atlantic	37.4 ± 4.4	30.3 ± 2.3	32.3 ± 3.4	0.4 ± 0.3	1.4 ± 0.9	1.9 ± 0.0	7.7 ± 0.9	2.5 ± 0.0	1.3 ± 1.5
	Indian	36.9 ± 4.4	30.7 ± 3.8	32.4 ± 6.1	0.5 ± 0.3	1.4 ± 0.1	1.8 ± 0.2	8.7 ± 1.8	3.5 ± 0.8	0.0 ± 0.0
	Pacific	31.6 ± 0.1	38.5 ± 0.3	29.9 ± 0.2	0.9 ± 0.1	1.8 ± 0.3	2.0 ± 0.1	5.6 ± 0.1	3.0 ± 0.1	0.7 ± 1.0
Digestive Gland	Atlantic	33.5 ± 1.5	33.5 ± 3.3	33.0 ± 2.1	0.7 ± 0.1	2.2 ± 0.3	1.8 ± 0.2	8.2 ± 0.8	2.9 ± 0.7	1.9 ± 0.5
	Indian	34.9 ± 2.4	30.1 ± 5.8	34.9 ± 3.7	0.6 ± 0.2	1.6 ± 0.4	1.6 ± 0.2	6.9 ± 2.1	3.8 ± 1.8	0.0 ± 0.0
	Pacific	31.9 ± 1.3	35.8 ± 2.2	32.2 ± 1.6	0.9 ± 0.1	1.7 ± 0.2	2.0 ± 0.2	6.1 ± 0.7	2.9 ± 0.6	1.7 ± 0.5

Supplementary Table 3.3: Eigenvalues, variation (%) cumulative variation (%) and loadings for principal component analysis of the fatty acid percentage composition data of *Euphausia superba*. Whole krill fatty acid profiles were taken from the total lipid, and stomach and digestive gland fatty acid profiles were taken from the neutral lipid fractions. Largest loadings for positive and negative components of each principal component are highlighted in bold.

Sample Type	**Whole Krill**				**Stomach**			**Digestive Gland**		
Principal component	PC1	PC2	PC3		PC1	PC2	PC3	PC1	PC2	PC3
Eigenvalues	0.344	6.4 e^{-2}	4.4 e^{-2}		2	0.861	0.193	1.06	0.378	9.5e^{-2}
% variation	65.9	12.4	8.4		62.4	26.9	6.0	64.0	22.7	5.7
Cumulative % variation	65.9	78.3	86.8		62.4	89.3	95.3	64.0	86.7	92.4
Loadings				**Loadings**						
14:0	0.173	**-0.530**	**0.521**	14:0	**-0.760**	**0.542**	0.119	**0.520**	**-0.726**	0.139
16:4n-1	-0.176	-0.198	-0.374	16:4n-1	-0.144	0.035	-0.012	0.033	-0.206	-0.122
16:1n-7c	**0.329**	-0.210	-0.296	16:1n-7c	0.032	0.140	**-0.273**	0.294	0.027	-0.289
16:0	0.105	-0.155	-0.073	16:0	0.040	0.107	0.081	0.101	-0.064	-0.072
18:3n-6	-0.109	0.008	0.175	18:3n-3	0.095	-0.151	0.072	-0.016	0.143	0.307
18:4n-3	**-0.724**	-0.386	0.010	18:2n-6	-0.114	-0.218	0.270	-0.117	-0.197	0.435
18:1n-9c	0.101	-0.103	-0.025	18:4n-3	-0.257	-0.112	-0.028	-0.426	-0.224	**0.535**
18:1n-7c	0.139	0.286	-0.128	18:1n-9c	0.133	-0.009	-0.265	-0.030	**0.206**	0.162
18:0	-0.021	-0.166	0.089	18:1n-7c	0.144	0.001	-0.206	-0.042	0.180	0.016
20:5n-3	-0.178	0.207	-0.223	18:0	**0.261**	0.128	**0.715**	-0.033	0.141	0.041
20:4n-3	0.106	0.312	0.083	20:5n-3	-0.269	-0.329	-0.270	**-0.450**	-0.282	**-0.482**
20:1n-9c	0.007	-0.131	**-0.386**	20:1n-9c	0.018	-0.021	-0.187	-0.133	0.080	-0.080
21:5n-3	-0.151	-0.013	-0.244	21:5n-3	-0.054	-0.086	0.052	-0.081	-0.094	-0.076
22:6n-3	-0.433	**0.374**	0.178	22:6n-3	-0.355	**-0.666**	0.179	-0.436	-0.348	-0.154
22:1n-9c	0.021	-0.211	-0.378	22:1n-9c	-0.056	-0.126	0.242	-0.126	-0.076	-0.107

APPENDIX 2

Additional Outcomes from this Research

Co-Authored papers:

- ERICSON, J. A., HELLESSEY, N., NICHOLS, P. D., KAWAGUCHI, S., NICOL, S., HOEM, N. & VIRTUE, P. 2018a. Seasonal and Interannual Variation in the Fatty Acid Content and Composition of *Euphausia superba* samples derived from the Scotia Sea fishery *Journal of Crustacean Biology,* 38**,** 662-672.

- ERICSON, J. A., HELLESSEY, N., KAWAGUCHI, S., NICOL, S., NICHOLS, P. D., HOEM, N. & VIRTUE, P. 2018b. Adult Antarctic krill proves resilient in a simulated high CO 2 ocean. *Communications Biology,* 1**,** 190.

- ERICSON, J. A., HELLESSEY, N., KAWAGUCHI, S., NICHOLS, P. D., NICOL, S., HOEM, N. & VIRTUE, P. 2019a. Near-future ocean acidification does not alter the lipid content and fatty acid composition of adult Antarctic krill. *Scientific reports*, 9, 1-10.

- ERICSON, J. A., HELLESSEY, N., NICHOLS, P. D., NICOL, S., KAWAGUCHI, S., HOEM, N. & VIRTUE, P. 2019b. New insights into the seasonal diet of Antarctic krill using triacylglycerol and phospholipid fatty acids, and sterol composition. *Polar Biology,* 1-12.

Presentations at:

- Antarctic Climate and Ecosystems Co-operative Research Centre (ACE CRC) Symposium 2016 , Hobart, Australia (poster)

- University of Tasmania (UTAS) Graduate Research Conference 2016, Hobart, Australia (poster)

- Science of Omega-3: Balancing the Scales, Omega-3 Centre Conference 2016, Sydney, Australia (poster)

- Homeward Bound Symposium at Sea 2016, Ushuaia, Argentina (oral, chosen as 1 of 78 voyagers out of over 3,000 applications globally)

- Association of Polar Early Career Scientists (APECS) Online Conference "Outside the Box" 2017, global webinar (oral)

- 3rd International Krill Symposium 2017, St Andrew’s, Scotland (oral)

- Scientific Committee for Antarctic Research (SCAR) Biology 2017, Leuven, Belgium (oral and poster)

- Australasian section of the American Oil Chemists Society (AAOCS) Conference 2017, Tanunda, Australia (oral)

- University of Tasmania (UTAS) Graduate Research Conference 2017 (oral – 3 Minute Thesis Finalist, top 10 from over 500 applicants)

- Marine Ecosystem Assessment for the Southern Ocean (MEASO) conference 2018, Hobart Australia (oral and poster – local organising committee)

- Scientific Committee for Antarctic Research (SCAR) and the International Arctic Science Committee (IASC) POLAR2018 conference, 2018, Davos, Switzerland

Visiting Researcher stay at:

- British Antarctic Survey, June 2017, with Dr Geraint Tarling

Science Communication and Outreach through UTAS, IMAS and CSIRO:

- TAstroFest (2016)

- Antarctic Festival (2016-2018)

- ABC radio interviews (2016-2019)

- Mercury, Examiner and the Advocate newspaper articles (2016-2019)

- Online guest blog posts and interviews (2016-2019)

- Young Tassie Scientists (2016-2019)

- STEM Professionals in Schools (2017, with Margate Primary and St Helen's Regional School)

- TV interviews (Four Corners - 2017)

- Science Investigation Awards Judge (2017-2018)

- Festival of Bright Ideas – IMAS (2017), WhySci and Young Tassie Scientists (2018)

- Tasmanian Youth Science Forum (2017-2019)

- Conoco Phillips Science Experience (2017-2019)

- DataTas Keynote Presentation (2018)

- RoboCup Junior Judge (2018)

- "Now that's what I call Science" radio show co-hosting at Edge Radio (May 2019)

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